University of Alberta

Effervescent Dry Powder for Respiratory Drug Delivery

by

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Dedicated with lots of love to:

My parents, Paulo and Vera Ely

My sister and brother, Juliane and Paulo Henrique Ely

The most important people in my life and who gave me strength to finish this project.

Abstract

The aim of this project was to develop a new type of respiratory drug delivery carrier particles that incorporate an active release mechanism.

Spray drying was used to manufacture powders containing polybutylcyanoacrylate nanoparticles and ciprofloxacin as model substances for pulmonary delivery. The carrier particles incorporated effervescent technology, thereby adding an active release mechanism to their pulmonary route of administration. Effervescent activity of the carrier particles was observed when the carrier particles were exposed to humidity.

For the effervescent formulation the MMAD was 2.17 μm +/- 0.42, FPF was 46.7 % +/- 15 and the GSD was 2.00 +/- 0.06. The effervescent carrier particles can be synthesized with an adequate particle size for lung deposition. This opens the door for future research to explore this technology for delivery of a large range of substances to the lungs with possible improved release compared to conventional carrier particles.

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List of Abbreviations

5-FU	5-Fluorouracil
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CLSM	Confocal Laser Scanning Microscopy
CO_2	Carbon Dioxide
DNA	Deoxyribonucleic Acid
DPI	Dry Powder Inhaler
DPPC	Dipalmitoylphosphatidylcholine
ED	Emitted Dose
FITC	Fluoresceine Isothiocyanate-Dextran
FPF	Fine Particle Fraction
GSD	Geometric Standard Deviation
HCl	Hydrochloric Acid
LBG	Locust Bean Gum
MMAD	Mass Median Aerodynamic Diameter
MS	Mucous Simulants
NaOH	Sodium Hydroxide
NH_3	Ammonium Hydroxide
PBCA	Polybutylcyanoacrylate Nanoparticles
PEG	Polyethylene glycol

PI	Polydispersity Value
pMDI	Pressured Metered Dose Inhaler
RNA	Ribonucleic Acid
SEM	Scanning Electronic Microscopy
SLS	Sodium Lauryl Sulfate
STB	Sodium Tetra Borate

CHAPTER 1

Introduction

1.1 – Overview of Lung Delivery

The pulmonary route of administration has been used for many years for the local treatment of lung diseases. Since the early 1990s, there has been an intensive growing interest in systemically delivering agents such as biotechnology derived proteins and peptides via the respiratory tract. Example of these includes anti-IgE and insulin, which are difficult to formulate orally (Labiris and Dolovich, 2003). Nowadays the major areas of pulmonary research are asthma (Hardy and Chadwick, 2000), cystic fibrosis (Garcia- Contreras and Hickey, 2002), tuberculosis (Pandey and Khuller, 2005; Zahoor et al, 2005) and lung cancer (Rao et al, 2003). Aerosol delivery for lung cancer has been investigated as a promising approach to local drug delivery. It started with the work of Slevchenko & Resnik, 1968 who examined the concomitant use of radiotherapy and inhalation aerosol chemotherapy. Recent advances in inhaled pharmaceutical delivery devices (dry powders inhalers, metered dose inhalers and nebulizers) have created renewed interest in human trials of aerosol chemotherapy, with Phase I clinical trials of doxorubicin (Placke et al, 2002). Nowadays, aerosol products for pulmonary delivery comprise more than 30% of the global drug delivery market (Pison et al, 2006).

Drug delivery to the lungs requires an aerosol vehicle, which consists of either aerosol droplets containing the drug, or powder particles of appropriate size for lung delivery. The distal lung has an enormous adsorptive surface area and a thin absorption mucosal membrane (Courrier et al, 2002). Drug absorption in the lungs occurs across the alveolar epithelium, which has a surface area of about 75 m^2 (Fuller et al, 2002; Wilkins et al, 2003). The alveolar region presents low thickness epithelial barrier, extensive vascularization and relatively low proteolytic activity compared to other routes of administration (Grenha et al, 2005). Local drug delivery presents several advantages in the treatment of these diseases over other routes of administration. Drug inhalation enables rapid drug deposition in the target organ and requires in most cases a lower dose (only one tenth to one fifth compared to an oral dose), which can result in fewer systemic side effects than others routes of administration (Brown et al, 2001; Feddah et al, 2000; Keller, 1999). Other advantages of pulmonary drug absorption are that it avoids first pass metabolism in the liver (Agu et al, 2001) and it offers the potential for needle free delivery (Bosquillon et al, 2001).

Inhalation therapy has some challenges to overcome to accomplish efficacious and safe drug delivery. The deposition of an aerosol to the lungs depends on its particle size. The major challenge is to achieve a suitable particle size for deep lung deposition. The appropriate drug dose must be deposited in the alveolar region to achieve therapeutic effectiveness and appropriate pharmacokinetics (Labiris and Dolovich, 2003). The success of inhaled particles depends mostly on their size and

density. The particle size is expressed in terms of mass median aerodynamic diameter (MMAD). It is well documented in the literature (Bosquillon et al, 2001, Dailey et al, 2003; Lucas et al, 1999) that aerosol particles must have an aerodynamic diameter of 1-5 μm for deposition in the alveolar region of the lung. Other challenges are:

a) Loss of compound during inhalation,

b) Dosing difficulties due to inefficient aerosolization in the inhaler,

c) Enzymatic degradation within the lung,

d) High production costs (Dailey et al, 2003).

The most common pulmonary delivery systems can be classified as dry powder inhalers (DPI), propellant-metered dose inhaler (pMDI) and nebulizers. Advances in dry powder inhalation technology and the known advantages of dry powders over solutions have made DPIs a very attractive drug delivery method.

The use of drug delivery systems like drug-loaded nanoparticles is of increasing interest in biomedical research. Drug delivery systems are used to overcome many obstacles in drug delivery such as drug absorption barriers, drug stability and lower systemic drug toxicity. However, most studies in the past have focused on the oral and intravenous route of administration for drug delivery systems. The first nanoparticle-containing intravenous drug delivery system was recently approved as medicine in the United States under the name Abraxane®. It contains albumin-bound paclitaxel for the treatment of metastatic breast cancer (Schaumburg,

Ill: Abraxis Oncology). The use of nanoparticles in pulmonary drug delivery is rather new and there are only few studies, which investigated the possibility of inhalable nanoparticles (Grenha et al, 2005, Sham et al, 2004; Tsapis et al, 2002; Zahoor et al, 2005).

1.2 - Pulmonary Delivery Devices

Pulmonary delivery of an active compound can be achieved using aerosols. They are an effective method of delivering therapeutic agents to the respiratory tract. Besides medically used gases (e.g. for anesthesia), there are three major pharmaceutical devices used for inhalation therapies: dry powder inhalers (DPIs), pressured metered dose inhalers (pMDI) and nebulizers (Finlay, 2001; Newman, 2004). The deposition of particles in the lungs is greatly influenced by the delivery system. A considerable number of inhalation devices are currently on the market however none of these devices has all the desirable properties of an optimal inhaler. Furthermore, in order to be effective and safe pMDIs, DPIs and nebulizers must have some important characteristics. These characteristics include (a) protection of the drug formulation from the ambient environment (b) dose a repeatable amount of drug each time activated (c) for powder formulation deagglomerate the powder into particles of a respirable size; or disperse suspensions or solutions into appropriate droplets (d) it must be portable and easy to use (Voss and Finlay, 2002; Wang et al, 2004).

1.2.1. Dry Powder Inhaler (DPI): A DPI is a breath-actuated metered-dosing system and does not need a propellant. Today it is considered one of the most promising techniques for delivering drugs to the lungs (French et al, 1996; Hess, 2000, Kotaro et al, 2003). The dry powder is delivered through a device to the lungs during a single breath. In a DPI, the medication by itself can be the dry powder or it is a powder blend between large carrier particles and the drug. Carrier particles are needed because of the highly cohesive nature of pure micronized drug powders (Newman, 2004). The inhalable drug dose can be packed inside a small capsule, a disk or a compartment inside the inhaler. This offers the capacity to provide a wide range of single doses per inhalation (multiple unit-doses). Due to the breath-actuation, a patient does not have to coordinate the activity of breathing with the activation of the inhaler. However it may be necessary for patients to inhale as hard as possible for optimal particle dispersion and maximal lung deposition (Newman and Busse, 2002). Further advantages are low susceptibility to microbial growth and suitability for either water soluble and insoluble drugs. However, the small particle size required to reach the alveolar region of the lung causes problems in powder processing (poor flowability) and redispersion (strong agglomeration and adhesion) (Irngantiger *at al*, 2004). Using conventional devices, approximately 12-40% of the emitted dose is delivered to the lungs with 20-25 % of the drug being retained within the device itself (Labiris and Dolovich, 2003). In order to solve these problems different strategies have been developed to improve the flowability within DPIs and make the powder to break up into individual particles. These strategies include the use of compressed air to desaggregate the drug particles in a spacer chamber and the preparation of large

and porous particles (Edwards et al, 1998). Such particles have lower inter-particle cohesiveness and are therefore superior for redispersion and aerosolization (Bosquillon et al, 2001; Edwards et al, 1998).

As mentioned earlier, a dry powder system requires often the use of a carrier particles (e.g. lactose) mixed with the drug to enable the drug powder to readily flow out of the device. However, the amount of these carrier substances can exceed the amount of the drug. In some blends the carrier fraction can represent 98% or more of the weight per inhaled dose (Wilkins et al, 2003). The particle size of the drug from these devices can range from 1 to 5 μm, but the particle size of the carrier particle can range from 20 to 65 μm. Most of the carriers are deposited in the oropharynx. Therefore, the drug must deagglomerate from the carrier particles to be deposited into the deep lung. Otherwise drug and carrier particles will be deposited in the upper respiratory tract and this will lower the respirable fraction. According to Wang et al, 2004 the dispersion of dry powders as aerosols during inhalation depends on a number of factors. These factors can be mainly divided into 1- intrinsic powder properties and 2- dynamic flow effects. An example for intrinsic properties is interparticle forces that are very important for the optimization of aerosols. The dynamic flow effects are important in deagglomeration and delivery of powders (Wang et al, 2004). An inhalation flow rate of 30 l min^{-1} is the minimum required flow rate to sufficiently desaggregate particles for the effective use of DPIs (Timsina et al., 1994). One disadvantage of using DPI is the need for high inspiratory flow. Patients must generate an inspiratory flow rate of an average of 40 to 60 L/min to

produce a respirable powder aerosol. Children younger then 5 years cannot develop flow at this rate. For this reason a device other than the DPI should be used for children younger than five years old (Wilkins et al, 2003).

1.2.2. Propellant-Metered Dose Inhaler (pMDI): A propellant metered dose inhaler was first introduced in 1956. Nowadays most inhaled drugs are administered using MDI for the treatment of asthma (Hardy and Chadwick, 2001; Steckel and Brandes, 2004). With low cost per dose and high dose reproducibility, pMDIs dominate the current market (Wang et al, 2006). pMDI is a pressurized inhaler that delivers medication by using a propellant (chlorofluorocarbons - CFC and more recently, hydrofluoroalkanes – HFAs) (Labiris and Dolovich, 2003). The principle consists in a high vapor pressure substance called the propellant that is contained under pressure in a canister. When released, it sends liquid propellant at relatively high speed from the canister. The vapor pressure of a solution is an important property since it serves as a quantitative expression of escaping tendency. It can be adjusted by mixing appropriate mole fractions of different propellants. According to Raoult's law in an ideal solution, the partial vapor pressure of each volatile constituent is equal to the vapor pressure of the pure constituent multiplied by its mole fraction in the solution:

$$\text{Eq (1.1)} \quad P_1 = iX_1P_1^0$$

Where P_1 is the vapor pressure of the solvent with added solute, X_1 is the mole fraction of solvent and P_1^0 is the vapor pressure of the pure solvent.

Pressurized pMDIs can contain dissolved drugs or micronized powder suspended in the propellant. The volume of the formulation and the amount of the drug released per actuation of the pMDI are a function of the size of the metering chamber. The metering chamber volume can vary from 30 to 100 µl. However, the exact amount of drug delivered to the patient is difficult to predict because of a high variability between inhalation techniques used by the patients (e.g. patient's breathing pattern, inspiratory flow rate and hand mouth coordination when using the inhaler) (Labiris and Dolovich, 2003; Wilkins et al, 2003). pMDIs used to deliver conventional micronized suspensions can be very inefficient, with not more than 10% to 15% of the dose deposited in the lungs. This is due to the fact that the majority of the dose is deposited in the oropharynx (Clark, 1995; Hirst et al, 2002). The distribution of the particles within the lungs can also be affected by diseases and therefore alter the pharmacokinetics and pharmacodynamic response of some devices (Agu et al, 2001; Schulz, 1998).

Pressurized MDIs are used to administer bronchodilators (Albuterol-Ventolin and Proventil), anticholinergics (Ipatropium Bromide – Atrovent), and steroids (Flucasone Propionate HFA – Flovent HFA). Nowadays the pMDI has received improvements as drug delivery devices and improved formulations disperse the drug more readily during inhalation (Keller, 1999).

1.2.3. Nebulizers: A nebulizer is the oldest method of aerosol delivery device. It has existed for over 100 years and it has been used for many years to treat asthma

and other respiratory diseases. There are two types of nebulizers: pneumatic nebulizers and ultrasonic nebulizers. Aerosol formation by nebulizers is achieved either by converting a liquid into a fine spray that uses a gas as the driving force (pneumatic nebulizers) or ultrasonically by the vibration of a piezoelectic crystal (ultrasonic nebulizers) (Sharma et al, 2001). These device converted drug solution into a continuous fine aerosol mist, normally 1-5 microns in size which can be inhaled directly into the lungs via a mouthpiece or face mask. It is recommended that aerosols generated by nebulizer be inhaled during quiet breathing. Fast inspiration promotes deposition in the upper airways, whereas slow controlled inhalation promotes pulmonary deposition and decreases upper airway deposition. Lung deposition from nebulizers is generally below 10% of the dose and devices delivering more than 15% of the dose to the lungs are rare. Another limitation of nebulizer is that some agents (e.g. proteins and peptides) are often very unstable in aqueous solution, and easy hydrolyzed. The process of nebulization also exerts high shear stress on the compounds, which can lead to protein denaturation (Agu et al, 2001).

1.3. Particle Size Distribution

The aerosol particle size is one of the most important variables in defining the distribution and deposition of a drug in the lung. It affects how much and where the aerosols will deposit in the respiratory tract. The particle size distribution can be often described by the log-normal distribution. The area under the log-normal curve represents the mass of drug in the distribution and only two parameters: MMAD

(mass median aerodynamic diameter) and GSD (geometrical standard deviation) are necessary to describe the particle size distribution of the true aerosol (Thiel, 2002). Another important factor is the flow rate (Sharma et al, 2001).

The particle size is expressed in terms of aerodynamic diameter. The aerodynamic diameter relates the particle to the diameter of a sphere of unity density that has the same settling velocity as the particle of interest regardless of its shape or density (Labiris and Dolovich, 2003). For spherical particles it is calculated based on the product of the mass median diameter of the powder and the square root of the true particle density.

$$\text{Eq (1.2) daer} = \sqrt{\frac{\rho}{\rho 1}} d$$

Where ρ is particle density, $\rho_1 = 1 g/cm^3$ and d is mass median particle diameter (Hinds, 1999; Rabbani et al, 2005). It can also be measured experimentally using devices such as cascade impactor methods.

The MMAD has 50% of the size below and above the mass median aerodynamic diameter. It accounts for particle aggregates and provides information on the ultimate aerodynamic behavior of an aerosol (Bosquillon et al, 2001). The knowledge of the aerodynamic diameter is fundamental for predicting the particle deposition in the lungs.

The geometric standard deviation is a measure of the variability of the particle diameter within an aerosol (Labiris and Dolovich, 2003). It is defined by the ratio of the diameters of particles from aerosols corresponding to 84% and 16% on the cumulative distribution curve of the weights of particles. For a log–normal distribution the GSD is calculated as follow:

$$\text{Eq (1.3) GSD: } \sqrt{\frac{SizeX}{SizeY}}$$

Where size X is the particle size for which the line crosses the 84% mark and size Y the 16% mark.

The use of GSD to describe the particle size distribution requires that the particle size is log-normally distributed (Courier et al, 2002). A GSD of 1 indicates a monodisperse aerosol, while a GSD $\geq$1.2 indicates a heterodisperse aerosol (Labiris and Dolovich, 2003).

Most aerosols used in pharmaceuticals field are composed of particles of different particle sizes ("polydisperse") and geometries (Kim, 2000). This causes deposition of different particle fractions in different parts of the respiratory tract. For the same MMAD, lung deposition of these aerosols (GSD $\geq$1.2) is expected to be greater than those of monodisperse aerosol (GSD = 1) (Kim, 2000).

1.3.1 Particle Deposition in the Respiratory Tract

Deposition is the process that determines what fraction of inspired particles will eventually be deposited in the respiratory tract. There are three major particle deposition mechanisms in the lung: inertial impaction, sedimentation and Brownian diffusion.

1.3.1.1. Inertial Impaction: It is the mechanism that plays a major role for deposition of an aerosol particle with a mass median aerodynamic diameter (MMAD) larger than 5 μm. This mechanism dominates the deposition in the extra thoracic and tracheo – bronchial regions when the velocity and mass (particle size and density) of the particles involve an impact on the airways. It happened at places where a relatively large particle has to change its course, such as at airway bifurcations or when the respiratory tracts are partially blocked (Courrier et al, 2002). The chance of impaction increases as the air velocity, particle size and particle density increases as well.

1.3.1.2. Sedimentation: It occurs in the alveolar region and concerns particles from aerosols with a MMAD ranging from 1 to 5 μm (Darquenne and Prisk, 2004). Sedimentation results from the action of gravitational forces on the particles. It is proportional to the square root of the particle size and is thus less significant for small particles. This mechanism of deposition depends on particle motion.

1.3.1.3. Brownian Diffusion: It is an important mechanism for aerosol particles with a MMAD equal or less then approximately 1 μm. Diffusion is the main mechanism of inhaled nanoparticles or fine particles due to displacement when they collide with air molecules (Obersdörster et al, 2005). Particles entering the airways may be displaced by the random bombardments of gas molecules and run up against the respiratory walls (Newman et al, 1982). Since it is caused by gas molecule collisions, the effectiveness of this mechanism increases as particle size decreases (Schulz, 1998). E.g. small particles may be inhaled but then exhaled right back out.

In summary, the deposition of aerosol particles in the respiratory tract is dependent on the aerodynamic particle size, breathing conditions (inhalation flow rate) and on the pulmonary delivery device. Particle can also change size, for example droplets may evaporate or particles may grow by condensation. In addition, there are clearly differences in particle deposition between adults and infants as well as between healthy and diseased lungs (Kim, 2000; Schulz, 1998). This is due to different airflow rates and different lung geometries.

1.4. Spray Drying

Spray drying has been recognized as a successful process to generate powders in a single step from solutions (Irgantiger et al, 2004). It is an important method for the dehydration of food fluids and is also extensively used in chemical and pharmaceutical industries. The main objective of spray drying is to remove a solvent

by evaporating. Typically, the spray drying is performed using an aqueous system, but it can also be undertaken with organic solvent-based solutions under controlled conditions. It consists out of the nebulisation of a solvent on suspension. The solvent evaporates during a short contact time with the stream of hot air when the product is spray dried. The temperature of the air stream can be a little above of the boiling point of the solvent. But, this does not mean that the product is exposed to this temperature due to the endothermic reaction when solvents evaporate. Spray drying allows control of particle shape, morphology and density, depending on the spray drying conditions (Steckel and Brandes, 2004).

The spray drying is compost of five basics steps:

1- Atomization of feed/water mixture into an aerosol (creation of droplets)

2- Mixing of droplets with the drying medium

3- Drying of solvent in a stream of hot air or dry gas

4- Separation of the dry product from moist gas

5- Cooling

The open cycle type is the most common type of spray dryer designs. It works with an atomized feed and drying air injected at the same time into a spray dryer chamber in the same direction. The particles are dried in the chamber as the atomized feed and hot air mix dry out the solvent and then the residual solids fall into the chamber base. Another type of spray dryer is the closed cycle system. In this case the

heating medium is recycled and reused, typically an inert gas such as nitrogen is used (BÜCHI Labortechnik AG - Training Papers Spray Drying).

Moreover, it is important to consider all properties of a powder product and the equipment when choosing a spray drying system. The properties of the final solid product depend on the atomization energy; feed properties, nozzle size, airflow and inlet and outlet temperatures (Elversson et al, 2002). These properties are described below:

Atomization energy - The most critical step in the spray drying process is to produce droplets of specific sizes and surface area by atomization. Atomization is the mechanism of changing a bulk liquid into aerosols particles. The degree of atomization, under a set of spray drying conditions, controls the drying rate, and therefore the required particle residence time, and the particle size. The nozzle is usually placed within the drying chamber and it is operated such that the spray has intimate contact with the drying air. The atomization is inversely proportional to the droplet size.

Feed properties – The feed pump is a device that transfers the solution to the atomizer. It affects the characteristics of the spray. For example, a coarser spray will be obtained and the final product will be composed of large particles when the viscosity of the feed solution is increased.

Nozzle size - There are different sizes of nozzles available with different spray characteristics allowing the machine to produce a desired product. For example, as

the diameter of the nozzle opening increases, the size of the droplet increases as well. In addition small nozzles will produce droplets of medium size and large nozzles will produce larger droplets.

Airflow - The spray flow rate is the amount of compressed air needed to disperse the solution, emulsion or suspension. Different gas besides compressed air can also be used. It is important to know that the higher the spray flow rate, the smaller the droplet size and it result in smaller particles in the final product.

Inlet and outlet temperatures – The temperature can be adjusted to evaporate the solvent and to allow the particle formation to complete in the drying chamber (Eerikäinem et al, 2004). Inlet temperatures are defined as the temperature of the drying air that flows through the instrument with the aid of the aspirator. A high inlet temperature will cause rapid evaporation leading to small droplets. The outlet temperature is the temperature of the air stream containing the solid particles before it enters the cyclone. This temperature can be used to determine the moisture content and also influences the structure of the carrier particles. These two kinds of temperatures, the inlet and outlet temperatures and the difference between both is one of the most important points in the spray drying process. It also contributes to the physical properties of the end product, such as particle size, shape, morphology and final moisture content.

Spray drying is a common method used for microencapsulation of drugs because of important characteristics such as reliability, reproducibility and possible control of particle sizes and drug release. It has the advantages of being a continuous

process that is easy to scale up and only slightly dependent on the solubility of drug. Besides the pharmaceutical field, spray drying is used for producing detergents, flavouring agents and synthesizing fertilizers (BÜCHI Labortechnik AG-Training Papers Spray Drying).

1.4.1. Spray Freeze-Drying

Freeze drying is also known as a lyophilization process. It is the process of atomizing a liquid to form droplets, freezing the droplets and ice subliming at low temperature and pressure (Constantino et al, 2000). Spray freeze-drying is an alternative for biological materials that often need to be dried to stabilize for storage or distribution and to enhance the physico chemical stability of colloidal vectors such as liposomes and nanoparticles. It is also the most complex and expensive form of drying.

Spray freeze-drying is a promising technique for producing small particles for lung delivery and an alternative for heat sensitive drugs. A study conducted by Maa et al, 1999 have shown that spray freeze–dried powders showed much better aerosol performance then the spray dried powders. In this study, Maa et al shows that spray freeze-drying is a more efficient process in terms of product recovery and product quality (Maa et al, 1999). However, some formulations or drugs might not be suitable to be produced by the freeze spray drying method.

1.5. The Respiratory Tract and the Clearance Mechanisms of the Lung

The respiratory tract can be divided into three major regions: the extra thoracic region also called the upper respiratory tract, the tracheo-bronchial region and the alveolar region (the deepest region of the lung), where both regions together form the lower respiratory tract (Newman et al, 1982; Wilkins et al, 2003).

The extra thoracic region consists of the oral cavity, the nasal cavity, the larynx and the pharynx (oropharynx and nasopharynx). The nose pharynx and larynx conduct respiratory gases to and from the lungs. These structures serve as frontline defense mechanism for the lungs (Wilkins et al, 2003). The extra thoracic region varies from individual to individual. For example, individual variations can occur in the shape of the oral cavity due to changes in the position of the tongue and jaws. Also, the laryngeal opening into the trachea changes shape with flow rate and so it is a time dependent geometry (Finlay, 2001).

The trachea marks the starting point of the conducting system, commonly called the tracheobronchial tree (Wilkins et al, 2003). The trachea is lined with ciliated columnar epithelium, which overlies a glandular vascular submucosa. The ciliated cells of the mucosa clear and defend the respiratory tract. The portion of the trachea within the thorax is affected by pressure differences across its walls (Wilkins et al, 2003).

The alveolar region is also called the parenchyma or pulmonary region and it includes all parts of the lung that contains alveoli, beginning at the respiratory bronchioles. Each respiratory bronchiole branches into the alveolar ducts. The ducts carry three or four spherical atria that lead to the alveolar sacs supplying 15-20 alveoli. Additional alveoli are located directly on the walls of the alveolar ducts and are responsible for approximately 35% of gas exchange. An adult human lung contains about 300 million alveoli, each measuring about 250 μm in diameter when expanded. Its volume is estimated to be $1.05 * 10^{-5}$ ml, and its air- tissue interface to be $27 * 10^{-4}$ cm^2. Based in these calculations it is assumed that the lung has a total volume of 4.8 L and a respiratory volume of 3.15 L and that the air- tissue alveolar interface is 75 m^2 (Fuller et al, 2002; Wilkins et al, 2003). All those values are based on measurements of a normal lung. Factors such as age, weight, height as well as diseases like asthma, cystic fibrosis; emphysema can affect and modify these characteristics (Wilkins et al, 2003). The alveolar surface area is large and allows a rapid systemic absorption of soluble drugs. The pulmonary circulation supplies the alveolar capillary membrane, whereas there is absorption into the bronchial circulation from the conducting airways. Alveolar macrophages are a normal resident of the alveolus and are avidly phagocytic. Those macrophages form an important defense mechanism against inhaled bacteria and are a major means of clearing the alveolus of inhaled dust. As phagocityc activity occurs predominantly with particles between 1-2 μm, it has been shown there is a decrease in phagocityc activity for sizes less than 1-2 μm making nanoparticles as an effective method for escaping the lungs natural clearance mechanism (Grenha et al, 2005). Two types of epithelial cells are

present in the alveolar region of the lung: the Type I and Type II pneumocytes (Smith, 1996). The Type I is a very large thin cell stretched over a very large area. They are responsible for gas exchanges occurring in the alveoli. The Type II granular pneumocyte are smaller, roughly cuboidal cells that are usually found at the alveolar septal junctions. This cell type is responsible for the production and secretion of lung surfactant. The lamellar bodies contain the phospholipid component of the surfactant.

1.5.1. Lung Surfactant: Function and Composition

Lung surfactant is an important component of the alveolar interface, which is crucial in maintaining the stability of the pulmonary system. In the absence of surfactant, the collapse of multiple alveoli rapidly progresses to severe respiratory distress (Weaver and Conkright, 2001),

Lung surfactant is a mixture of proteins and surfactants – specific proteins synthesized by specialized type II pneumocytes in the lungs. This system counterbalances the elastic fibers of the lung and the wall tensions of the alveoli. It also helps the lung from collapsing during expiration. Additionally, it stabilizes the gas exchange region of the lung by reducing the surface tension at the air – aqueous interface of the alveoli (Banerjee, 2002; Schűrch et al, 1999). Another function is particle transport from the alveoli to the ciliated airways by a surface tension gradient. Particles deposited in the airspace of the lung are wetted and displaced towards the epithelium by the surfactant film during the retention process (Schűrch et al, 1999).

The surfactant is composed of 90% in weight of lipids and 10% proteins. More than 85% of the surfactant lipids are phospholipids with content of 40-80% of dipalmitoylphospatidylcholine (DPPC) (Courrier et al, 2002). DPPC is the major component responsible for the reduction of surface tension. It forms a semi-crystalline monolayer that is able to reduce the surface tension near to zero, on compression of the monolayer (Banerjee, 2002).

In addition to the phosphatidylcholines, there are negatively charged phosphatidylglycerols, accounting for 10-15% and other anionic lipids and cholesterol present in the surfactant. The 10% left is composed of 4 main specific proteins: the hydrophobic protein SP–B and SP-C and the hydrophilic proteins SP-A and SP-D. SP–B and SP-C play a role in the optimal functioning of the surfactant film and the other two participate in host-defense mechanism of the lungs against inhaled pathogens and stimulation of the pulmonary macrophages (Banerjee, 2002; Courrier et al, 2002; Krol et al, 2000).

1.5.2. Lung Clearance Mechanism

The lungs have a local immune system as a first line of defense in protecting the body against pathogens from outside and inside of the body. The main cells of this immune system are alveolar macrophages and lymphocytes. Studies have shown that macrophages are the predominant cell type of the pulmonary immune system (Rao et al, 2003). The macrophages internalize pathogens and infections agents such as virus,

bacterias and parasites by the process of phagocytose (Ahsan et al, 2002). They are also important components of the body's frontline defense against tumor cells (Ahsan et al, 2002).

Drugs deposited in the alveolar region might be phagocytosed and cleared by alveolar macrophages or absorbed into the pulmonary circulation (Newman et al, 1982). The success of the macrophage encountering the particles appears to be facilitated by chemotactic attraction of alveolar macrophages to the site of particle deposition (Oberdörster et al, 2005). Therefore, the uptake of particles by macrophages is size dependent (Asgharian et al, 2001). Particles with a diameter of less than 0.26 μm are minimally taken up by macrophages (Courrier et al, 2002). Insoluble particles are phagocytes by macrophages with most of the particles being engulfed within a few minutes. However they can reside for days before being completely removed by macrophages depending on their size and shape (Davies and Feddah, 2003; Daviskas et al., 1995). Insoluble particles can either be cleared by the lymphatic system or moved into the ciliated airway currents in alveolar fluid and then cleared via the mucociliary escalator. The clearance rate by macrophages is slower compared with mucociliary clearance (Davies and Feddah, 2003).

There are two different mechanisms for pulmonary clearance for particles deposited between the larynx and the terminal bronchioles. Insoluble particles are trapped in the mucus and are moved toward the pharynx and ultimately to the gastrointestinal tract by the upward movement of the mucus generated by the

metachronous beating of the cilia (Labiris and Dolovich, 2003). Insoluble drugs particles that are inhaled but not removed by the mucociliary escalator are taken up by intraluminal macrophages, transported to the interstitium and removed by lymphatic drainage. Mucociliary clearance is affect by diseases such as immotile cilia syndrome, bronchiectasis, cystic fibrosis and asthma (Houtmeyers et al, 1999).

1.5.3. Systemic Elimination

The drug that is absorbed via the lung membrane or gut after clearance by the mucociliary escalator and escapes the first pass metabolism becomes systemically available. At this time the drug is in the systemic circulation, it behaves like an intravenous dose and is eliminated by hepatic and renal clearance. Systemic accumulation of an inhaled substance will occur if the dosing interval is shorter than the time required for its elimination.

1.6. Nanoparticles

Over the past few years there has been a growing interest in developing biodegradable nanoparticles, and using them as an effective drug delivery device. Nanoparticles were first proposed as drug delivery systems in the late 1970s (Kreuter, 1978). They are solid colloidal particles ranging from 1 to 1000 nm. Nanoparticles consist of macromolecular materials, which have a large (functional) surface area (Borm and Kreyling, 2004). They can be used as adjuvant in vaccines, or as drug

carriers, in which the drug is dissolved, entrapped, bounded, encapsulated and/or to which the active principle is adsorbed or attached (Borm and Kreyling, 2004; Kreuter, 1991; Soppimath, 2001).

Nanoparticles have been used to deliver drugs to a target organ and their use presents several advantages compared to the use of conventional dosage forms, for example: increased efficacy, decreased toxicity of side effects and overcome multi drug resistance (Soppimath et al, 2001). *In vitro* and *in vivo* studies have shown the high clinical potential of nanoparticles based carriers to be used for poorly soluble drugs, to overcome multi-drug resistance or as carriers for selective delivery of oligonucleotides to tumor cells (Brigger, 2002). Nanoparticles can also be characterized as vehicles for the controlled and targeted ophthalmic, oral, and intravenous as well as implanted delivery of vaccines and drugs. Advantages of drug specific delivery are that it can be tailored to avoid uptake by organs such as the liver, spleen and other parts of the reticuloendotelial system (Illum, 1987). It may also be tailored to target diseased lesions or tumors (Branon-Peppas and Blanchette, 2004). Nanoparticles can be coated by some materials. Studies have shown that the coating of nanoparticles can enhance the drug delivery through the blood brain barrier (Alyautdin et al., 1997; 1998; 2001; Schroeder et al., 1998; Gulyaev et al, 1999) and alter the body distribution after an intravenous injection (Araujo et al, 1999).

To synthesize nanoparticles a large range of polymers have been used in drug delivery as they can effectively deliver the drug to a target site (Soppimath et al,

2001). Today there are several different biocompatible polymers available to manufacture nanoparticles and this offers the option of changing the surface characteristics or modifying the surface functionality. Some examples are serum albumin (Wartlick et al, 2004), polycyanoacrylates (Sommerfeld et al, 1998), polylactic-co-glycolic acid (Pandey et al, 2003) chitosan (Grenha et al, 2005) and gelatin (Azarmi et al, 2006). Polyalkylcyanoacrylate nanoparticles have been shown to be a potential drug carrier system for tumour targeting due to their biodegradability, biocompability and their capacity to modify the tissue distribution of a variety of anti-tumour agents (Douglas et al, 1986).

To prepare nanoparticles a large range of methods can be found in literature. Soppimath et al, 2001 gives a good review on the preparation of biodegradable polymeric nanoparticles, including the polymers mentioned above. The methods can vary depending on, for example, the type of polymer (polymerization of monomers), and the type of solvent (evaporation or solvent diffusion methods). Another important factor is, if the drug will be entrapped, encapsulated or attached. It is very common that researchers modify the methods of preparation according to their needs and expectations.

1.6.1. Nanoparticles and Lung Delivery

Pulmonary drug delivery offers local targeting for the treatment of respiratory diseases and it is also an option for the delivery of drugs systemically (Pison et al,

2006). Nanoparticles have been proposed for pulmonary administration to achieve a locally drug levels in the lungs via the aerosol route. They exhibit certain characteristics that make them ideal for pulmonary drug delivery and for treating lung specific diseases such as lung cancer. Cellular uptake studies have shown that cells like cancer cells and epithelial cells are able to take nanoparticles up (Ghirardelli et al, 1999; Huang et al, 2002; Russel–Jones et al, 1999). As demonstrated by Ramesh et al cationic DOTAP: cholesterol nanoparticles successfully delivered tumor suppressor genes to primary and disseminated lung cancers with minimal toxicity. More recently Ramesh et al, 2004, evaluated nanoparticle-mediated delivery of the human *mda*–7/IL-24 gene to primary and disseminated lung tumors *in vivo*. The results showed a suppression of the tumor growth in primary as well as in metastatic tumors. In addition tumor vascularization was decreased in *mda*–7/IL-24–treated tumors (Ramesh et al, 2004).

Due to their small size, nanoparticles avoid unwanted mucociliary clearance and phagocytic clearance (Grenha et al, 2005) by remaining in the lung lining fluid until dissolution (Tsapis et al, 2002) or translocation by the epithelium cells (Oberdörster et al, 2005). Studies using inhaled nanoparticles dispersed in aqueous droplets suggest that the mucus clearance can be overcome by nanoparticles, possible due to the rapid displacement of the particles to the airway epithelium via surface energetics (Schurch et al, 1990). As mentioned earlier, *in vivo* studies using nanoparticles (Araujo et al, 1999) have demonstrated that a protective coating of a carrier system can prevent or greatly reduce uptake by the mononuclear phagocyte

system which is very important for pulmonary delivery due to presence of macrophages in the lungs. In addition nanoparticles with lipophilic coatings are better phagocytosed than their hydrophilic counterparts (Ahsan et al, 2002).

One issue with pulmonary nanoparticle delivery is that their small size limits their lung deposition. Aerosolized nanoparticles larger than 50 nm in diameter have only very limited sedimentation, inertial impaction or diffusion, which causes them to be predominantly exhaled from the lungs after inhalation (Finlay, 2001). The major consequence of this would be a significant loss of drug due to exhalation.

Nanoparticles must be produced with special care and many times requires the use of a carrier particle (Sham et al, 2004). Carrier particles are a dissolvable matrix usually made out of lactose or mannitol. By incorporating nanoparticles into a carrier particle the ability of an inhaled particle to deposit in the lungs is higher compared to a nanoparticle formulation alone ($\geq$50 nm in diameter). According to Grenha et al, 2005, particles, which will entrain in the respiratory tract and be deposit in the lung, depend on the aerodynamic diameter of the particle. After deposition in the lung, the carrier matrix made from carbohydrates is expected to dissolve quickly and releases the nanoparticles which then promote the absorption of the associated therapeutic macromolecule (Grenha et al, 2005).

As demonstrated by Sham et al, 2004 nanoparticles can be incorporated into lactose carrier particles and these particles can be made with an appropriate MMAD

for deep lung deposition. This approach can be used for the local delivery of drug-loaded nanoparticles to the lungs (Sham et al, 2004). In addition, Azarmi et al, 2006 have shown that doxorubicin loaded nanoparticles carried by dry powders increased the cytotoxic effect in two lung cancer cell lines. This study indicated that doxorubicin-loaded nanoparticles are more potent than the free drug (Azarmi et al, 2006).

Nanoparticles offer the potential for sustained drug release throughout the alveolar space. A sustained release formulation will release the drug over an extended period, which may enhance the control of diseases states, for example, asthma (Cook, 2005). The release rate of nanoparticles depended on some factors such as surface bound/adsorbed drug, diffusion through the nanoparticles matrix and nanoparticle erosion or a combined release (Soppimath et al, 2001). In addition to the sustained release the formulations needs to protect the active moiety and deliver the drugs at predetermined rates. The formulations must be stable in the inhaler as well being compatible with the lung environment (Hardy and Chadwick, 2000).

Because of these special characteristics and advantages, nanoparticles are very promising as a drug carrier. They might make it possible to target many different sites within the human body.

1.7. 5-Fluorouracil (5-FU)

5-fluorouracil (5-FU) is a fluorinated pyrimidine analog belonging to the antimetabolite class of agents. It has a fluorine atom in the 5 position of the uracil molecule.

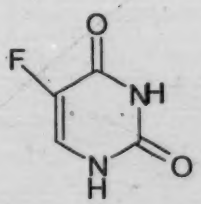


Figure 1.1 – Chemical structure of 5-Fluorouracil

5-fluorouracil is one of the most extensively used antitumor drugs. Over the past years it has been used against a large number of tumour types including breast cancer, gastric and colon cancer, head and neck cancer, and esophageal and anal cancer (Presant et al, 2002).

Pharmacological studies have shown that most of the drug is catabolized in the liver to 5, 6-dyhidro fluorouracil, fluoroureido acid and finally to alpha-fluoro-beta-alanine. A small fraction of the administered 5-FU is transported into the tumor cells, where it is anabolized to various fluorinated nucleosides. These anabolites interact with thymidylate synthetase and are incorporated into RNA and DNA. Additional mechanism of action includes direct incorporation into RNA to

interfere with RNA transcription and, to a lesser extent, direct incorporation into DNA.

The pharmacokinetics of 5-FU is characterized by a rapid distribution phase and a rapid elimination phase resulting in a short plasma half-life, in the range of 10-20 minutes (Ackland et al, 1997). 5-FU follows a two compartment pharmacokinetic model with a large volume of distribution (Nassim et al, 2002). Approximately 15% is excreted intact in the urine, 90% of which is excreted in the first hour. The liver and gastrointestinal mucosas are the primary sites of metabolism.

5-FU was chosen as a model drug for this project because it is a common drug used to treat different types of cancer. In this study, 5-FU was incorporated into polybutylcyanoacrylate nanoparticles and the drug loading efficiency was investigated. Some of the formulations prepared in our lab were investigated in cell culture. The objective of using 5-FU nanoparticles for lung delivery was to decrease the systemic side effects of the drug when administered orally and to improve its therapeutic index. Therefore it may offer a better treatment alternative to patients that are undergoing chemotherapy.

1.8. Ciprofloxacin

Ciprofloxacin is a potent and broad–spectrum fluoroquinelone that is effective against a number of microorganisms, mainly gram-negative bacterias (Conley et al, 1997). It is structurally related to nalidixic acid, the prototype 4-quinolone antibiotic. It belongs to the second generation of quinolones. Some examples that are included in the second generation are norfloxacin, enoxacin, levofloxacin, nadifloxacin and ofloxacin (Hopper and Rubinstein, 2003).

Ciprofloxacin has a fluorine atom at the 6-position, a piperazine moiety at the 7-position and a cyclopropyl ring at the 1-position. Enhanced gram-negative *in vitro* activity is associated with the presence of the piperazine group at the 7-position; presence of this group may enhance the penetration of the quinolones into bacteria and may also be responsible for the increased activity against *Pseudomonas aeruginosa.* A fluorine attachment at the 6-position is thought to broaden the spectrum and increase the antimicrobial activity up to 30 times. The 2-carbon group at the 1-position is essential for antimicrobial activity. Ciprofloxacin has a cyclopropyl attachment, which may be spatially equivalent to a 2-carbon group and is thought to enhance overall antibacterial activity. Ciprofloxacin hydrochloride (CPFX-HCl) is the pharmaceutical formulation, structure of which is shown in figure 1.2.

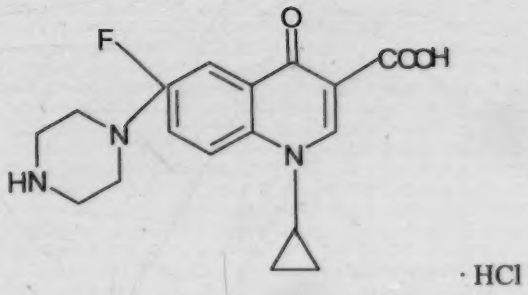


Figure 1.2. Chemical structure of ciprofloxacin (1, 4-dihydro-1-cyclopropyl-6-fluoro-4-oxo-7- (1-piperazinyl) - 3-quinolinecarboxylic acid).

Ciprofloxacin exert an inhibitory effect on bacterial DNA gyrase. It leads to the termination of chromosomal replication and to interference with cell division and gene expression. This action appears to be responsible for the bacterial activity of this agent. A secondary mechanism is the inhibition of the activity of topoisomerase IV that leads to the separation of two united DNA molecules and subsequent interference with cellular replication. Ciprofloxacin has been shown to have a superior ability to penetrate most tissues compared to other antibiotics, it accumulates in macrophages and neutrophils and it is bactericidal in low pH environments. Oral and intravenous forms of ciprofloxacin have been used to treat respiratory tract infections. However ciprofloxacin administered intravenously or orally has a relatively unfavorable pharmacokinetic profile in the lower respiratory tract. This includes a relatively short elimination half life of 1.0 to 1.6 h and a low area under the concentration-time curve (AUC) of 43 to 113 mg.h /liter (Conley et al, 1997). There is also increasing evidence

that with repeated use organisms become resistant and the drug should not be given more frequently than every three months (Hodson and Geddes, 2000).

Ciprofloxacin has been used in lower respiratory tract infections and urinary tract infections. It is also approved as an inhaled form to threat anthrax after an individual has been exposed. An inhaled form of anthrax infection happens when spores of *Bacillus anthracis* are inhaled, and they germinate and the bacterial cells infect the lungs and spread to the lymph nodes in the chest

1.8.1. Respiratory Tract Infections in Patients with Cystic Fibrosis

Cystic Fibrosis is a genetic disease resulting from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). CFRT is present in the apical membrane of airway epithelial cells. It has been shown to be the channel through which chloride is secreted into the lung lumen (Smith, 1997). Although the genetic disorder affects epithelial cells or multiple organs, the respiratory tract is it associated with the most significant morbidity. Lower respiratory tract infection with *Pseudomonas* spp. and/or *Staphylococcus aureus* is a major cause of morbidity in patients with cystic fibrosis (Hooper and Rubinstein, 2003) and respiratory failure is responsible of death in over 90% of the cases (Hodson and Geddes, 2000). Ciprofloxacin has been the most extensively studied drug in patients with cystic fibrosis due to its potency against *Pseudomonas aeruginosa.* Antibiotic therapy has been shown to be an important factor in improving the survival in patients with cystic

fibrosis. Pharmacokinetic studies have indicated that despite significant mal absorption in CF patients, ciprofloxacin still absorbed well.

As mentioned early, ciprofloxacin is widely used as a type of treatment used to fight respiratory infections. In this project ciprofloxacin was incorporated into lactose carrier particles and a new type of carrier particles that was developed. The incorporation of ciprofloxacin into the carriers might help the delivery of the drug to the alveolar region of the lungs. The new type of carrier particles might also support the dispersion and dissolution of ciprofloxacin in the lungs and increases its efficacy.

1.9. Effervescent Technology:

Effervescent preparations have been utilized in oral drug delivery for more than 200 years. Since that time, a large number of preparations utilizing effervescent technology have been produced including stomach distress medications, vitamin supplements, and analgesics (Eichman and Robinson, 1998). An effervescent tablet or powder can allow a large dose of ingredients to be taken in a single serving. Clinical studies performed on a variety of effervescent products demonstrate that the ingredients penetrate into the blood stream within as little as fifteen minutes. After swallowing, conventional solid tablets or capsules are transported to the stomach where they disintegrate gradually. The passage takes variably long in different people, depending on anatomical and physiological factors. In the case of a longer passage time, solid dosage forms can dissolve partially and this can cause irritation of

the mucous membranes. With a dissolved effervescent tablet, the ingredients are evenly distributed in the solution, so that high, localized drug concentrations can not occur. The solution of the effervescent tablet contains the salts of an acids and carbonates. These preparations are partially or fully soluble in water. The drug dissolution is accelerated by the released carbon dioxide when the organic acid and the carbonate react.

A very simple reaction can cause the production of gas that is responsible for the effervescent effect:

$$\text{Acid} + \text{carbonate} \rightarrow CO_2 + \text{Acid Salt} + H_20$$

Mechanism of action: An acid is used to neutralize a carbonate. This reaction releases carbon dioxide gas. Water is needed to start the reaction. Without the water, neither the acid nor the carbonate can dissociate.

In general, higher ratios of acid to carbonate will yield in a faster reaction. It will also assure that the carbonate is completely reacted. If the ratio of the acid is not stoichiometrically balance to the carbonate then some carbonate will be left unreacted. In general, a 1:1 weight ratio of acid to total carbonates is common. However, highly reactive, highly soluble systems may use acid to carbonate ratios as lows as 1:10. Examples of acids are: Citric acid, Ascorbic acid, Tartaric acid, Adipic acid and Malic acid.

The effervescent formulation that is generally used in tablets for oral administration was applied for the first time, in this project, to the pulmonary route. In this work, polybutylcyanoacrylate nanoparticles and ciprofloxacin hydrochloride hydrate were used as two different model substances for pulmonary delivery. Drug release and dispersion of nanoparticles were separately compared using lactose carrier particles that dissolve without effervescent reaction, to the new effervescent carrier particle.

2.0. Mucous and Cough

Mucus is a secretion of the lining of various membranes in the body. It is a viscous colloid containing antiseptic enzymes and immunoglobulins. Mucus is produced by goblet cells in the mucous membranes that cover the surfaces of the membranes. It is composed of ~1% by weight of salts and other dialyzable components, 0.501% free protein, a similar proportion of carbohydrate–rich glycoproteins (also called mucins) and ≥95% of water (Houtmeyers et al, 1999).

Cough is the way that the body finds to remove foreign material or mucus from the lungs or throat. There are two classifications of cough: 1 – the productive coughs that produces phlegm (phlegm is a type of mucus) or mucus from the lungs and 2- the nonproductive coughs (dry and not producing any mucous or phlegm). A cough is usually characterized by three factors: an inspiration of approximately

2.5 liters of air, the closing of the glottis and a quick rise in the intrapleural pressure to ~100 Torr (King et al, 1985).

In this study the effervescent powder was tested using a cough machine to determine if the powder has properties on the mucus and on the cough. A cough machine was designated to mimic the flow rates and time pattern of a real cough.

CHAPTER 2

Hypothesis and Objectives

Based on the previous discussion, the following hypothesis was established for the current work:

- Effervescent powders can delivery nanoparticles and drugs to the alveolar region of the lungs and improve drug delivery and drug dissolution.

To test this hypothesis, a number of objectives were identified:

- A new type of carrier particles that uses effervescent technology has to be formulated and applied to the pulmonary route of administration.
- Effervescent powders with appropriate mass median aerodynamic diameter (MMAD) have to be synthesized.
- The fine particle fraction (FPF) has to be improved for better lung deposition compared to normal lactose carrier particles.
- A new carrier system which has an active release mechanism needs to be developed and optimized.
- To demonstrate that is possible to produce an effervescent carrier system that is a superior drug delivery system for nanoparticles and drugs compared to conventional lactose carriers.

CHAPTER 3

Materials and Methods

3.1. - Preparation of Unloaded Polybutylcyanoacrylate Nanoparticles (PBCA)

3.1.1. Materials

Butylcyanoacrylate was a gift from Loctite Ltd (Dublin, Ireland), Dextran 70 (~70 kDa), Fluorescein isothiocyanate-dextran (FITC-Dextran) and Diethylaminoethyl – dextran hydrochloride was obtained from Sigma Chemical Co (St. Louis, MO, USA). 5-Fluorouracil solution was obtained from Mayne Plasma (Montreal, ON, Canada) and solid 5- FU was obtained from Sigma Chemical Co. All chemicals were of analytical grade and used as received.

3.1.2. Methods:

The butylcyanoacrylate nanoparticles were prepared by a standard procedure described by Scherer et al, 1993. This procedure is an anionic polymerization of butyl-2-cyanoacrylate monomer in an acidic medium (Sommerfeld et al, 1998). 100 µl of the monomer was slowly added by pipette to 10 mL of HCl 0.01 N solution, containing 0.100g Dextran 70.000 as a stabilizer. The polymerization occurred spontaneous. It was carried out under stirring (600 rpm) with a glass coated stirring

bar, at room temperature for 4 h. The resulting colloidal suspension was then adjusted to pH between 5 and 7 using 1 N NaOH.

3.1.3. Preparation of Fluorescent Polybutylcyanoacrylate Nanoparticles (PBCA)

Fluorescent nanoparticles were produced as described previously, but using 0.09g of Dextran 70.000 and 0.01g of fluoresceine isothiocyanate-dextran 70.000 (FITC). Nanoparticles were protected from light through the polymerization process.

3.2. Purification of Nanoparticles

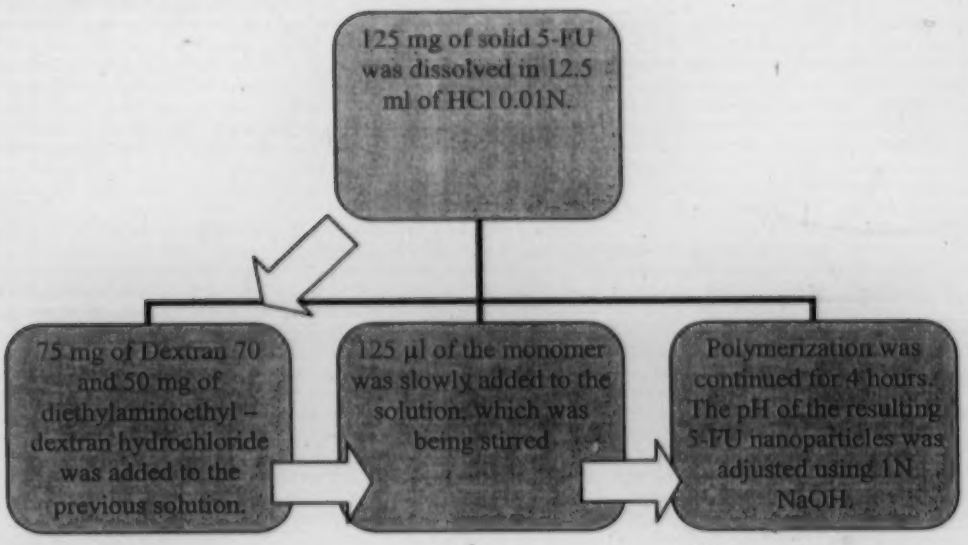
Nanoparticles were purified from unbound dye or other synthesis byproducts by centrifugation at 20.000-x g (Beckman Model J2-21) for ten minutes. After centrifugation the supernatant was removed and the nanoparticles were resuspended in 1mL of sterilized water. The particles were purified by three cycles of centrifugation and redispersion in fresh water medium. After the last centrifugation the fluorescent–labeled nanoparticles were stored at 4° C and protected from the light.

3.3. Preparation of 5- Fluorouracil Polybutylcyanoacrylate Nanoparticles

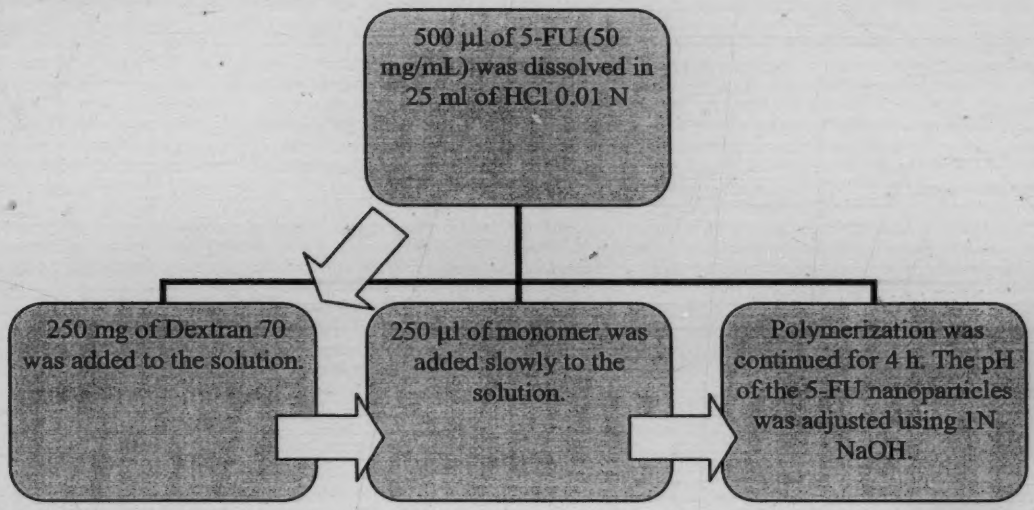
3.3.1. Methods

Five different methods based on the emulsion polymerization method (Sommerfeld et al, 1998) were used to prepare 5-FU nanoparticles. 5 - FU loading nanoparticles was carried out during the polymerization process.

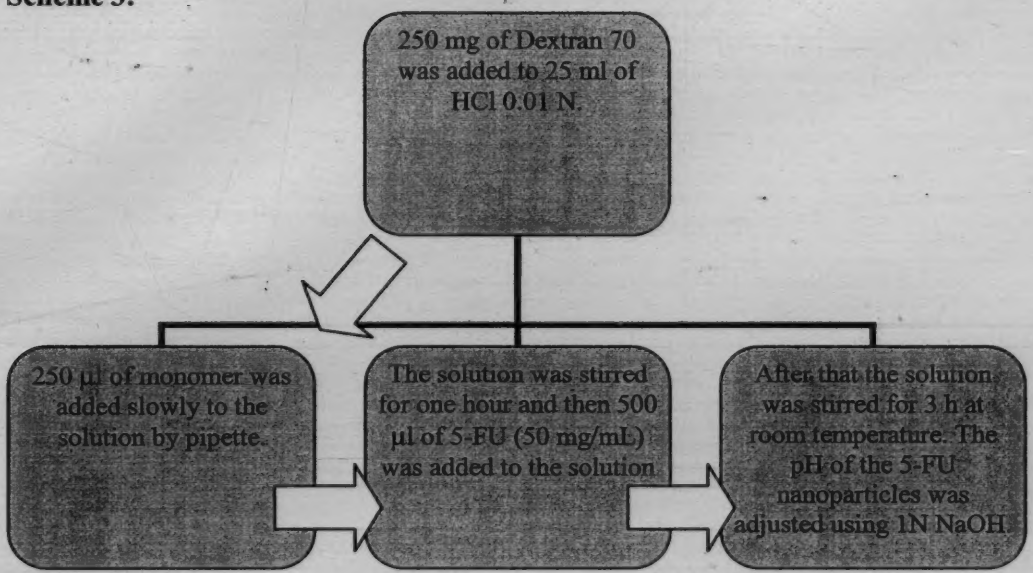
Scheme 1:


125 mg of solid 5-FU was dissolved in 12.5 ml of HCl 0.01N.
75 mg of Dextran 70 and 50 mg of diethylaminoethyl – dextran hydrochloride was added to the previous solution.
125 µl of the monomer was slowly added to the solution, which was being stirred
Polymerization was continued for 4 hours. The pH of the resulting 5-FU nanoparticles was adjusted using 1N NaOH.

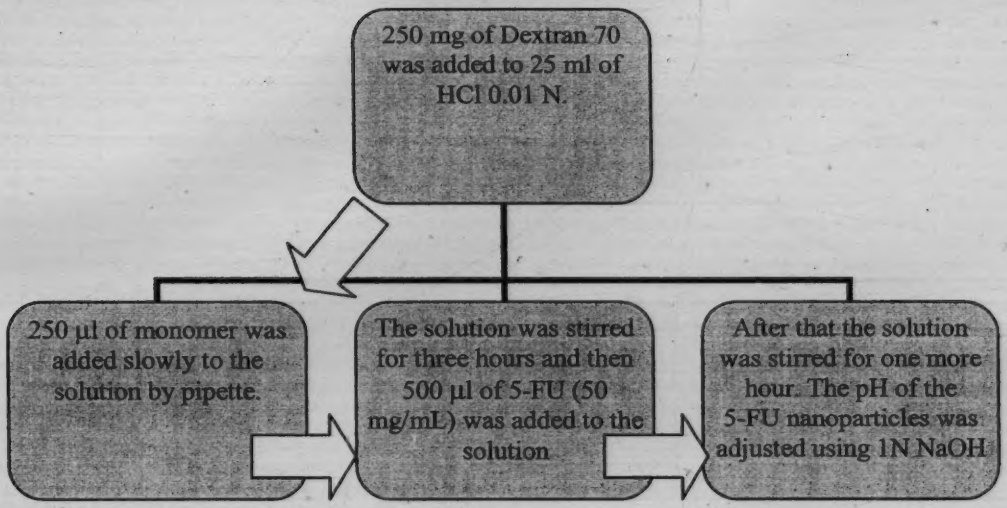
Scheme 2:


500 µl of 5-FU (50 mg/mL) was dissolved in 25 ml of HCl 0.01 N
250 mg of Dextran 70 was added to the solution.
250 µl of monomer was added slowly to the solution.
Polymerization was continued for 4 h. The pH of the 5-FU nanoparticles was adjusted using 1N NaOH.

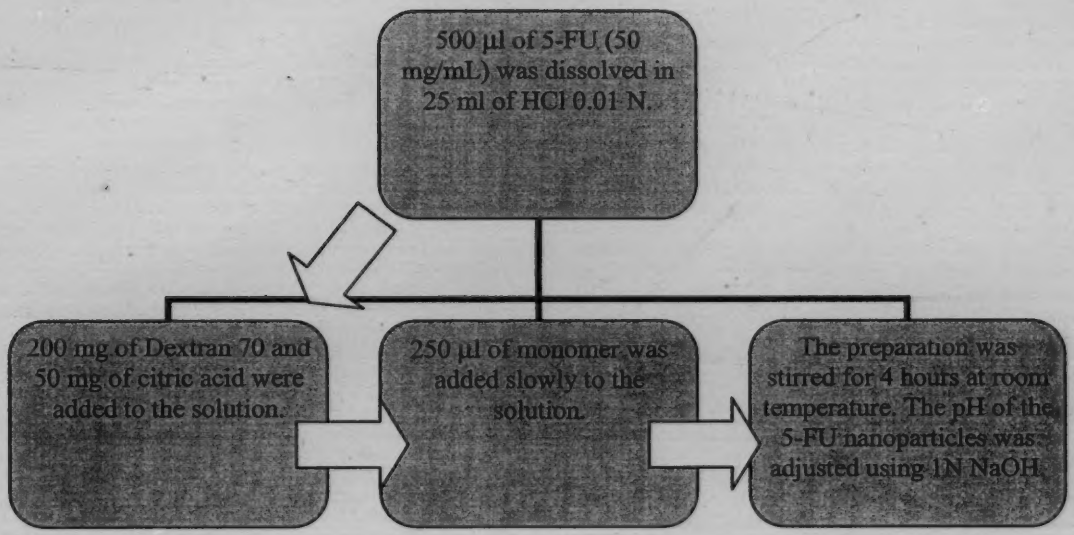
Scheme 3:


250 mg of Dextran 70 was added to 25 ml of HCl 0.01 N.
250 µl of monomer was added slowly to the solution by pipette.
The solution was stirred for one hour and then 500 µl of 5-FU (50 mg/mL) was added to the solution
After that the solution was stirred for 3 h at room temperature. The pH of the 5-FU nanoparticles was adjusted using 1N NaOH.

Scheme 4:


250 mg of Dextran 70 was added to 25 ml of HCl 0.01 N.
250 µl of monomer was added slowly to the solution by pipette.
The solution was stirred for three hours and then 500 µl of 5-FU (50 mg/mL) was added to the solution
After that the solution was stirred for one more hour. The pH of the 5-FU nanoparticles was adjusted using 1N NaOH

Scheme 5:


500 µl of 5-FU (50 mg/mL) was dissolved in 25 ml of HCl 0.01 N.
200 mg of Dextran 70 and 50 mg of citric acid were added to the solution.
250 µl of monomer was added slowly to the solution.
The preparation was stirred for 4 hours at room temperature. The pH of the 5-FU nanoparticles was adjusted using 1N NaOH.

The addition of 5-FU increased the pH of the HCl 0.01 N solution in all the above methods. In order to readjust the pH, 30 µl of 1N HCl was added to the solution.

3.3.2. Determination of Nanoparticles Loading Efficiency

Analytical determinations of 5-Fluorouracil concentrations were made using a UV spectroscopy (SPECTRONIC 3000 ARRAY–Milton Ray) at 267 nm. The standard curve was first established. It was generated by dilution of a freshly prepared stock solution of 5-FU (1 mg/ml) in distilled water. The solution was further diluted in order to obtain at least 5 concentrations and their absorbance values were detected. The correlation coefficient for the calculated linear regression was 0.9999 and the equation used was $\text{Abs} = 2.3209 \times A - 0.0944$.

Evaluation of 5-FU was determined by centrifugation of 5–FU loaded PBCA nanoparticles at 20.000 x g for 30 minutes. The supernatant was separated from the pellets. 100 µl of supernatant was diluted in 5 mL of distilled water. Drug loading was calculated from the difference between the initial 5-FU concentration and the 5-FU determined in the supernatant liquids (Gelperina et al, 2002). Free drug remains in solution and the incorporated drug sediments down with the nanoparticles. Supernatant 5-FU concentrations were calculated using the standard curve.

Loading efficiency was calculated as follow:

$$\text{Eq (3.1) \% Loading} = \frac{\text{Total amount of 5-FU in the supernatant}}{\text{Total amount of 5-FU added}} \times 100$$

3.3.3. Cell Culture

H460 (HTB-177) and A549 (CCL-185) lung cancer cell lines were used for the cell uptake studies. These cells are adherent cells, which grow as a monolayer. For the propagation of H460 cells ATCC, complete growth medium (RPMI 1640 medium with 2mMl-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10mM HEPES, and 1.0mM sodium pyruvate, 90%; fetal bovine serum, 10%) was used. For the propagation of A549 cells ATCC complete growth medium (Ham's F12K medium with 2mM l-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 90%; fetal bovine serum, 10%) was utilized. The cells were grown in 75mL flasks (Corning, Acton, MA, USA) in an atmosphere of 5% CO_2 and 100% relative humidity and sub cultured two to three times per week.

3.3.4 Cell Uptake Study

Approximately 10,000 H460 or A549 cells were grown in a Lab-Tek II Chamber Slide (Nalge Nunc, USA). After 24 h, when the cells were attached to the surface of the slides as a monolayer, the cell culture medium was removed and rinsed using PBS solution. The cells were incubated with

100 microliter of 5-FU-nanoparticle suspension for 1 hour. Then the nanoparticles were rinsed out using PBS. The cells were fixed by adding 0.5mL of 4% solution of paraformaldehyde in PBS for 10 min. This solution was then removed and 20 microliter of 1:9 PBS-glycerol containing 1 microliter of 1 mg/mL solution of DRAQ5 in PBS was added as mounting medium. Control samples were prepared by incubating cells with free 5-FU (5-FU injection) in PBS under the same conditions.

3.4- Development of Lactose Based Carrier

3.4.1. Materials

Lactose monohydrate was obtained from Wyndale (Kapuni, New Zealand). L-leucine, ammonium hydroxide, citric acid and rhodamine 6G were obtained from Sigma Chemical (St. Louis, MO, USA). Sodium carbonate anhydrous was obtained from BDH Inc. (Toronto, ON, Canada). Sodium bicarbonate was purchased from Caledom (Georgetown, ON, Canada). Polyethylene glycol (PEG) 6000 was obtained from Fluka Chemika-Biochemika (Buchs, Switzerland). T- MAZ 80 Polysorbate 80 was purchased from BASF (Ludwigshafen, Germany). 316 Silicone Release Spray was purchased from Dow Corning (Midland, MI, USA). Cargille, oil, Type DF, SPI, was obtained from West Chester PA.

3.4.2. Methods

2.5 % Polyethylene glycol 6000 (PEG 6000): 2.5 g of polyethylene glycol was dissolved in 100 mL of distilled water and the solution was heated and stirred for about two minutes to help on PEG dissolution.

2.5 % L-leucine: 2.5 g of L-leucine was dissolved in 100 mL of distilled water. According to the literature L-leucine has a limited solubility in water. The literature reports to be 2.2g/100g H_2O (Glinski et al, 2000) and for this reason it was used in our solutions about twenty drops of 0.5M NaOH were used to facilitate L-leucine's dissolution and solubility.

7g of lactose monohydrate were used to prepare the spray-dried samples. The lactose was added to 100 mL of distilled water. 5 mL of both polyethylene glycol 6000 and L-leucine solution were added to the lactose solution. The solution was heated and stirred for five minutes in order to accelerate the dissolution of lactose. The solubility of lactose at room temperature is 1g in 4.63 mL of water (Harjunen et al, 2002).

3.5. Development of Effervescent Carrier Particles

To establish a suitable formulation for the carrier particles different concentrations of lactose, carbonates, citric acid, lubricants, surfactants, ethanol and

water were used (see table 3.1). The ingredients were weighed. 10 ml of ammonia was added to the mixture of powders before the addition of water or other solvent. Ammonia was used to increase the pH of the solution to inhibit an effervescent reaction prior to spray drying. A titration was made in order to determine the amount of ammonia necessary to achieve the right pH. The pH was kept about 8.0 to 9.0.

3.5.1 Ingredients used at the Spray Drying Process and Examples

	Ingredients used	Concentration tested (%) or mL	Others Examples
Carbonates	Sodium Carbonate	0.75 % – 1.5%	L-lysine carbonate, Sodium glycine carbonate, Sodium carbonate, Magnesium carbonate, Calcium carbonate, Sodium hydrogen carbonate
Bicarbonate	Sodium Bicarbonate	0.75%	
Acid	Citric Acid	1.2%	Citric acid, Glycine citrate, Fumaric acid, Adipic acid, Malic acid, Lactic acid
	Ammonia	10 – 30 mL	
Lubrificants	L-leucine	0.8 -1 %	
	Polyethylene glycol 6000	0.8 -1 %	
Alcohols	Ethanol	10- 30%	
Surfactants	Polysorbate 80	1%	Polysorbate 85 (Tween 85)
	Sodium Lauryl Sulfate		Polyethylenepolypropylene glycol polymer (Pluronic F68)

Table 3.1 Ingredients used at the spray drying process and examples.

3.6. Spray Drying:

A Büchi 190 Mini–Spray Dryer (Büchi Laboratoriums-Technik, Flawil, Switzerland) was used to produce lactose and effervescent carrier particles. The diameter of the nozzle was 0.7 mm. 100 mL of lactose or of the effervescent solution was pumped into the feeding system of the spray dryer at room temperature. The resultant powder was collected in a container. Immediately after their collection, the powders were stored in vials and kept in a desiccator over silica gel.

Spray drying is the principle of separating a solid from a solution by forced evaporation. The solvent is evaporated by a stream of heated air, which transforms the small droplets in to a dry powder. The resultant powder is separated from the air in the cyclone separator and collected in a container.

The adjustable process parameters include inlet and outlet temperature, pump, airflow nozzle, and the aspirator partial vacuum. Figure 3.1 shows the spray dryer used to produce the lactose and the effervescent carrier particles.

Figure 3.1 Büchi 190 Mini–Spray Dryer

In order to obtain a formulation with appropriate size distribution different settings for each parameter were tested (see table 3.2).

Parameters	Range of control in the machine	Control tested
Inlet temperature	It does not show	90-180 °C
Outlet temperature	It does not show	50-130 °C
Atomizer air flow rate	600-800 (NormL/hr)	600-800 (NormL/hr)
Feed rate (pump)	0-20 (mL/min)	2-5 (mL/min)
Air flow rate (dial setting)	0-20	5-20
Heating rate (dial setting)	0-20	7-20

Table 3.2 Spray dryer parameters and controls used

3.7. Incorporation of the Nanoparticles into Carrier Particles

3.7.1 Methods

0.6 mg of Rhodamine 6G was added to 100 mL of effervescent solution prior to spray drying in order to have fluorescent-labeled carrier particles and to increase their visibility for the confocal microscopy. 7 mL of a suspension containing polybutylcyanoacrylate nanoparticles were added to either a 7% lactose solution, or to a 7% lactose solution containing PEG 6000 and L-leucine, or to an effervescent formulation solution or to an effervescent formulation solution containing PEG 6000 and L-leucine (5mL of 2.5% l-leucine and 2.5% PEG). The solutions were spray dried at temperatures between 120-140 °C and the glass chamber was shielded from light. After collection, the carrier particles containing nanoparticles were stored in vials covered with aluminum foil to protect it from light. The vials were kept in a desiccator over silica gel.

3.7.2. Nanoparticles Size and Size Distribution

Particle size distribution and the polydispersity index of polybutylcyanoacrylate nanoparticles were performed using a photon correlation spectroscopy (HSA3000, Malvern Instruments, UK). In this instrument, the polydispersity is equal to the intensity - weighted variance divided by the intensity-weighted average of the diffusion coefficient distribution squared. For the particle

size analysis, 3 ml of fresh filtered (0.45 μm) water was filled into a disposable cuvette. An aliquot of approximately 100 μl of suspension containing nanoparticles was added to the cuvette. Samples were sonicated for one minute immediately prior to measurement. To measure the size of the nanoparticles, the Zetasizer uses a laser beam, which is scattered by the nanoparticles in suspension. Measurements were carried out at room temperature at a light-scattering angle of 90°. The Zetasizer accumulates data from ten measurements and the average of these data is used to calculate the nanoparticles size. The particle size and the polydispersity of the polybutylcyanoacrylate nanoparticles were calculated based on the average values of ten measurements.

3.7.3. Nanoparticles Size Analysis

The size of the nanoparticles was measured before and after spray drying. To measure the size of nanoparticles after spray drying an adequate amount of both lactose and effervescent powder containing nanoparticles were dissolved in distilled and filtered water and sonicated for two minutes immediately prior measurements. In that way it was avoided that gas bubbles interfered with the measurement or that particles aggregated during the measurement.

3.7.4. *In vitro* Aerosol Deposition

The pulmonary deposition of the effervescent carrier particles was investigated *in vitro* using a Mark II Andersen Impactor (Thermo Andersen, Smyrna, GA) in combination with a new high efficiency inhaler developed by Finlay and Wang, 2002. This inhaler is responsible for deagglomeration of the powder. It utilizes a cyclone flow and mechanical impaction to disperse powders particles, directs the inhalation flow through a fine mesh located at the chamber outlet, and reduces the mouth-throat deposition via the use of a fine mesh that decreases the swirling flow and particle velocity by flow redirection and velocity dissipation (Wang et al, 2006). Figure 3.2 shows the structure of the inhaler.

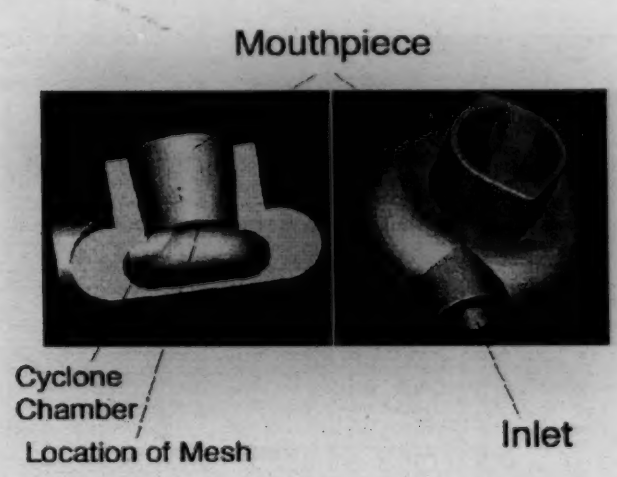


Figure 3.2 Structure of the inhaler.

The Andersen Impactor is a multi–stage designed to measure the size distribution and mass concentration levels of liquid and solid matter. It uses inertial

impaction to measure the size distribution of the powder that enters it (Voss and Finlay, 2002). The impactor has eight stages for particle size determination. Each stage gives a cut-point based on aerodynamic diameter of the particle. Figure 3.3 shows the Andersen Cascade Impactor used to perform the experiments.

Figure 3.3 Andersen Cascade Impactor

About 30 minutes before starting the Impactor, the plates were sprayed twice with 316 silicon spray grease to avoid fine particles from bouncing on the plates and also to reenter in the air-stream. If this happen it could give an incorrect size distribution (Vaughan, 1989). It was given fifteen minutes drying time after each application. The powder samples were weighted approximately between 0.0015 and 0.0020 g using a Sartorius 1207 MP2 analytical balance, which contains four decimal places. One single puff with the powder was introduced into the impactor. Each impactor-plate and the inhaler were weighted before and after dispersion of the powder. The plate of number zero was weighted as well in a three decimal places analytical balance TR-403 Denver Instrument Company. Nichols et al, 1998

published the calibration of the Andersen at the flow rate of 60 l/min. When this flow rate is applied the cut points of Andersen impactor is different from 30 l/min (standard flow rate) and from 90 l/min. A flow rate of 60 l/min is more representative of human inspiratory flow rates in typical dry powders inhalers (Finlay, 2001). The following table shows the cut points applied to perform the MMAD calculations for these experiments and also the cut points used when a flow of 30 l/min is applied.

Plate number	Aerodynamic diameter - 30 l/min- cut point (μm)	Aerodynamic diameter - 60 l/ min - cut point (μm)
0	9.0	5.6
1	5.8	4.3
2	4.7	3.4
3	3.3	2.0
4	2.1	1.1
5	1.1	0.51
6	0.7	-
7	0.4	-

Table 3.3 cut points applied to perform the MMAD calculations for the cascade impactor experiments (Voss and Finlay, 2002).

3.7.5. Mass Median Aerodynamic Diameter (MMAD):

The mass median aerodynamic diameter was measured using an Andersen Cascade Impactor in order to evaluate the possibility for use as an inhalant and to predict where the particles would be deposited in the respiratory tract. The MMAD was calculated by a nonlinear regression fit of a log-normal function to the data.

3.7.6. Geometrical Standard Deviation (GSD)

Geometric standard deviation is a measure of the spread of the distribution (Thiel et al, 1997). The use of a GSD to describe the particle size distribution requires that the particle size must be log-normally distributed (Courier *et al*, 2002).

To calculate the GSD, a nonlinear least squares analysis with a log-normal function was used.

3.7.7. Fine Particle Fraction (FPF) and Emitted Dose (ED)

Fine Particle Fraction means the particle fraction that can be delivered to the lungs. In our lab the FPF is defined as the fraction of loaded powder that is collected on plates 1-6 (e.g. aerodynamic diameter $\leq$5.6 μm, flow rate 60 l/min). The Mark II Andersen Cascade Impactor was used to determine the fine particle fraction of the dry powders. The emitted dose was calculated as the amount of loaded powder minus the amount of powder which was collected in the Andersen Cascade Impactor.

3.7.8. Scanning Electron Microscopy (SEM)

The lactose and effervescent powders were sprinkled onto a stub with silicon from a sticky tab. The unbound powders were dusted out by an air gun. The samples were coated with gold sputter using a S150B Sputter Coater (BOC Edwards, Crawley,

West Sussex, UK) and examined by scanning electron microscope (S2500 SEM, Hitachi, Tokyo, Japan).

3.7. 9. Confocal Laser Scanning Microscopy (CLSM)

The geometric diameter of the spray dried powders and the distribution of the nanoparticles through to the carrier particles were investigated using a Zeiss LSM 510 confocal laser-scanning microscope (Oberkochen, Germany). This model can collect twelve bit images using four detectors for fluorescent signals and a transmission detector for bright field images. It has four lasers with multiple laser lines for excitation of fluorophores (Argon, HeNel, HeNe2, and UV). The LSM 510 Software, version 2.01 was used to control the microscope and to analyze the data. The carrier particles were labeled with a red fluorescent label and the nanoparticles with a green fluorescent label. Small amounts of the powders were dispersed in immersion Cargille, oil, Type DF, SPI on glass slides and visually observed. The samples were observed before and after being exposed to humidity. To expose the powders to humidity a small amount of air from a mouth was delivered to the samples. The oil phase prevented any contact of humidity with the particles during the observation of the images. The particle morphology (porous vs. solid) was investigated by imaging different layers of the carrier particles. The powder particle sizes from selected samples were manually measured using the software Metamorph (v. 5.0, Universal Imaging Corporation). The mean powder size was calculated based on these measurements.

3.7.10. Water Content:

Water content was determined using 5 to 6 grams of the effervescent powder. The sample was dried in a vacuum oven over 48 hours at temperature of 40 C°. The loss on drying was calculated after 48 hours. The samples were weighted before and after drying.

The loss of drying was calculated as follow:

$$\text{Eq (3.2)} \quad \frac{\text{Weight of sample before drying} - \text{weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

3.8. Preparation of Ciprofloxacin Carriers

3.8.1. Materials

Ciprofloxacin hydrochloride hydrate was obtained from US Biological (Swampscott, MA, USA).

3.8.2. Methods

A formulation which was prepared of lactose (4g); sodium carbonate (5g), citric acid (4g), ammonia (10 mL) and water (100 mL) were used to produce effervescent carrier particles. In addition 5 mL (solution 2.5% in water) of polyethylene glycol and 5 mL of L-leucine (solution of 2.5% in water) was added to the solution. The solutions were prepared before and then added to the aqueous ammonia solution. 100 mg of ciprofloxacin hydrochloride hydrate was weighted and dissolved in 20 mL of HCl 0.01 N and added to the effervescent solution. The solution was pumped into the drying chamber at a rate of 2-mL/min. The inlet temperature was established at 120 or 130 °C, the outlet temperature depended on the inlet temperature and the liquid and gas flow rate, but for these values it was about 100-105 °C. After finishing the process the powders were collected and stored in vials, and kept in a desiccator over silica gel. The powders were stored in a desiccator over silica gel in order to avoid a Milliard reaction and to increase the stability of the powders.

3.8.3. Determination of Ciprofloxacin Loading Efficiency

Following impaction, each impactor plate from the Andersen cascade impactor and the inhaler were washed with 5 ml of distilled water. The resulting solutions containing the powder from the plate were kept in centrifuge tubes. The solutions were analyzed for ciprofloxacin content using UV spectroscopy at $\lambda = 271$

nm (SPECTRONIC 3000 ARRAY – Milton Ray). The UV spectroscopy determined the total amount of the drug as well as the amount of ciprofloxacin deposited on each stage. In addition, 15 mg of the effervescent or lactose powders were dissolved in 100 ml of water. Before the measurements, some selected samples were filtered (0.22 μm).

A stock solution containing 1 mg/ml of ciprofloxacin hydrochloride hydrate in distilled water was prepared. A standard curve was generated by dilution of a freshly prepared stock solution and further diluted in five known concentrations and analyzed using UV spectroscopy. The values were extrapolated from a standard curve by using known ciprofloxacin amounts. The correlation coefficient for the calculated linear regression was 0.9999 and the equation Abs = 96.39 A – 0.0024 was used to determine the dissolved drug content.

3.9. Cough Machine Experiments

3.9.1. Materials:

Locust bean gum, Sodium Tetraborate and Coomassie blue were purchased from Sigma Chemicals (St. Louis, MO, USA).

3.9.2. Methods:

Gels with rheological properties similar to respiratory tract mucous were prepared from locust bean gum (LBG) and it was called mucous simulants (MS). MS vary from a non-viscous and a non- elastic substance, to a MS with different degrees of viscosity and elasticity. An example of a substance with non-viscous and non-elasticity is water.

Preparation of 0.5% of locust bean gum (LBG): 100 ml of Ringer solution was heated to 80 ºC. 0.5 mg of LBG powder was added slowly to the hot solution while stirring to prevent it from clumping together. Then the solution was cooled to room temperature. 0.15 mg of Coomassie blue per 100 ml was added to the solution and it was stored in the fridge.

Preparation of a 0.01 M sodium tetraborate solution: 3.814 g of STB was dissolved in 100 ml of distilled water.

Preparation of mucus simulants with viscous-only: 0.5% locust bean gum (LBG) was added to a ringer solution.

Preparation of mucus simulants with viscous and elasticity: to create elasticity different amounts of sodium tetraborate solution were added to the LBG solution. As more sodium tetraborate was added the solution increased elasticity and viscosity.

3.9.3. Cough Machine Preparation

The cough machine system (figure 3.4) is composed of six elements. An 8-liter Plexiglas tank equipped with a Wilmot Castle pressure gauge served as a reservoir of pressured gas and simulated the capacitative function of the lungs and smaller airways. The gas release was controlled by an Asco solenoid valve located at the start of the outflow line. A constructive device was attached to the outflow of the solenoid valve. It was composed of short lengths of rubber tubing whose number and size were adjusted to the flow rates and the time course on the simulated cough. This is comparable to those conditions of an actual human cough (King et al, 1985). The artificial cough flow was set to a pressure of 8 psi, which corresponds approximately with the amount of pressure generated by a normal adult cough. This experiment was performed at Dr. Malcom King's Laboratory at University of Alberta.

3.9.4. Sample Preparation

The weight of the sample was determined by weighting the removable muzzle end-piece from the cough machine before and after the experiment on an analytical balance. The cough machine was placed about 40 cm from a paper made target. The paper-target (figure 3.5) was used to collect the drops of MS when discharged from the cough machine behave.

Figure 3.4 Cough machine

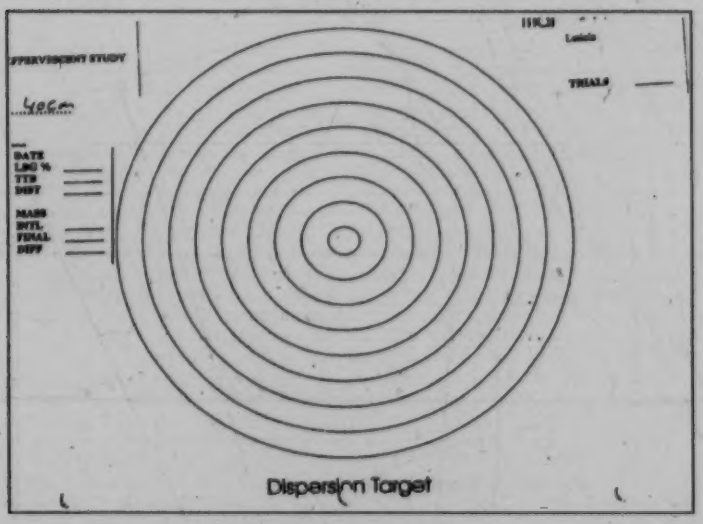


Figure 3.5 Paper target used to perform the cough machine experiments

3.9.5. Experiments

In the first experiment viscous MS without elasticity was used. Its compositions vary from 0.5% to 1% LBG solution. The sample was placed into the cough machine. Each experiment was repeated three times for each concentration.

The second experiment was performed in the same way. However, at this time MS with viscosity and elasticity was used. Different amounts of sodium tetraborate were added to 0.5% and 1% LBG solution.

In the third experiments effervescent powder formulations were added to a mucus solution. The solution was centrifuged for about 30 minutes or until the powder was totally mixed with the mucus. The powders were tested using viscous and elastic MS.

3.10 – Statistical Analysis

A paired t-test was used to compare the sizes of nanoparticles before spray drying into powders and after release from dissolved powders at the statistical *P*-value of 0.05.

CHAPTER 4

Results and discussion

4.1 Preparation of Nanoparticles

The first goal of this project was to synthesize cyanoacrylate nanoparticles with defined size range and a narrow size distribution. Another objective was to use nanoparticles for drug loading as well as to incorporate them into a new type of carrier particle for deep lung deposition. This carrier particle has an active release mechanism and is able to release nanoparticles or drugs actively.

After the introduction of nanoparticles as drug carriers in the late 1970s, (Kreuter, 1978), many studies described methods of preparation, distribution and potential targets. Polybutylcyanoacrylate nanoparticles were chosen for this project because they are well established and well documented in the literature and suitable as drug carriers (Alyaudtin et al, 2001; Kreuter, 1994; Simeonova et al, 2004; Sommerfeld et al, 1998). Furthermore, the polyalkylcyanoacrylate nanoparticles have been shown to be a potential drug carrier system for tumour targeting (Douglas et al, 1986).

4.2 Characterization of Nanoparticles

4.2.1. Particle Size and Size Distribution of Nanoparticles

The size distribution was determined using photon correlation spectroscopy (Zetasizer). According to the literature, nanoparticles have a particle size ranging from 1 to 1000 nm (Kreuter, 1994; Simeonova et al, 2002). The particle size distribution can be interpreted in three different ways: a) intensity b) volume and c) number (see table 4.1). The intensity model is the most accurate one and it was used in our measurements. The volume and number models are converted from the results obtained in the intensity model. The table below shows an example of nanoparticles that were investigated. The example in the table was synthesized by an anionic polymerization of butyl-2-cyanoacrylate monomer in an acidic medium and measured directly after synthesis.

Size (nm)	Intensity	Volume	Number
38.9	0.0	1.4	10.4
52.1	0.5	5.4	29.3
69.6	2.4	9.7	31.2
93.1	6.0	11.1	17.7
124.6	11.3	10.0	7.1
166.6	17.1	8.0	2.4
222.9	21.2	6.9	0.8
298.2	21.0	14.5	0.5
398.8	15.0	21.2	0.4
533.4	5.5	11.0	0.2
713.5	0.0	0.8	0.0

Table 4.1 Output of the measurement of the particle size distribution from the Zetasizer

Several samples of nanoparticles were produced and their sizes were measured. The average size of the polybutylcyanoacrylate nanoparticles produced in our lab was 153.55 +/- 33.14 nm (n = 10). The smallest size achieved was 108.4 nm and had a polydispersity value (PI) of 0.342. The polydispersity index is the ratio of the weight average molecular weight divided by the number average molecular weight. It is used to give the researcher an idea of the width of the molecular weight distribution (http://www.malvern.co.uk). The largest size of the nanoparticles was 214.8 nm and had a PI of 0.184.

4.2.2. 5-Fluorouracil Polybutylcyanoacrylate Nanoparticles

The average diameter of the unloaded polybutylcyanoacrylate nanoparticles determined by photon correlation spectroscopy was 153.55 +/- 33.14 nm (n = 10) and 113.15 +/- 15.6 (n = 6) nm for 5-FU nanoparticles. There was no statistical difference at $P < 0.05$ between the sizes of unloaded nanoparticles and the nanoparticles containing 5-FU. In contrast to other drugs, the addition of 5-FU did not increase the size of nanoparticles but rather decreased the size of the 5-FU nanoparticles.

The drug loading study showed that approximately 89.87 +/- 1.40 % of 5- FU was loaded on the polybutylcyanoacrylate nanoparticles. These results were an average of the encapsulation efficiency of all methods applied to produce 5-FU nanoparticles. These results were in accordance with the other published data (Simeonova et al, 2003 and 2004).

Our results have shown that the time when the polymer was added did not interfere with the drug loading efficiency. Drug loading of all methods tested achieved similar results with the exception of the sample that contained diethylaminoethyl–dextran hydrochloride. In this case the analysis did not detect any 5-FU.

Method	Amount of drug added (mg)	*Absorbance* value	Encapsulation efficiency (%)
1	125 *	Not detected	Not detected
2	25	0.989	88.56
3	25	0.948	89.5
4	25	0.904	91.85
5	25	0.938	89.6

* Addition of diethylaminoethyl–dextran hydrochloride

Table 4.2– Encapsulation efficiency of 5-FU nanoparticles

Results from table 4.2 are in agreement with the results found by Simeonova et al, 2004. He and his colleagues found an encapsulation efficiency of 85% of the total 5-FU added. The encapsulation efficiency results obtained in our lab are slightly higher than the one found by Simeonova (Simeonova et al, 2004). The encapsulation efficiency is one of the key criteria for evaluating the nano encapsulation process (Mu et al, 2005).

One of the objectives of this study was to determine whether the 5–FU loaded nanoparticles maintained their cytotoxic effect. Free 5-FU, 5-FU loaded nanoparticles

and blank nanoparticles were tested to evaluate their cytotoxicity. This was done using two different human non-small cell lung carcinoma cell lines, H460 and A549. The experiments were performed at the Cross Cancer Institute. Surprisingly the results obtained showed no cytotoxic effect on the lung carcinoma cells lines. However a study conducted by Dr. Shirzad Azarmi using 5-FU polybutylcyanoacrylate nanoparticles (nanoparticles were synthesized in our lab) showed cell uptake when investigated using Confocal Microscopy. DRAQ-5 was used for staining the nucleus of the cells. DRAQ-5 is a cell permeable anthraquinone, designed for use in a range of fluorescence detection technologies, in the discrimination of nucleated cells. Figure 4.1 shows the positive control for 5-FU solution. Figure 4.2 shows the uptake of 5-FU nanoparticles in A-549 lung cancer cells.

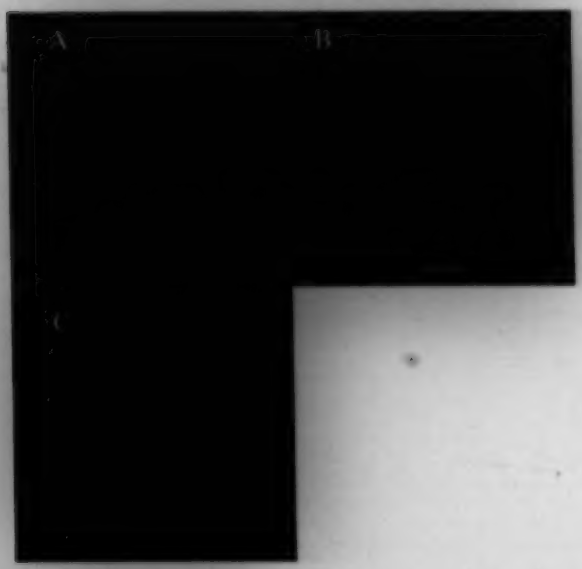


Figure 4.1 5-FU solutions

A) 5-FU detection B) DRAQ-5 detection C) Overlay of A and B

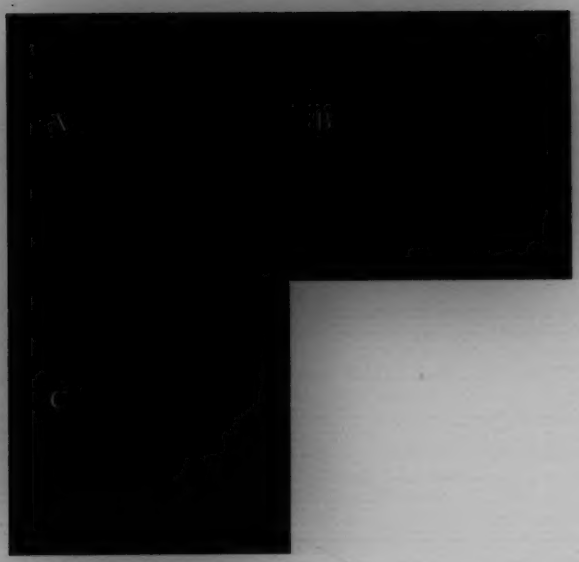


Figure 4.2 Uptake of 5-FU nanoparticles in A-549 lung cancer cells.

A) 5-FU detection B) DRAQ-5 detection C) Overlay of A and B

4.2.3 Discussion

Polybutylcyanoacrylate nanoparticles were produced and they were required to have a narrow size distribution. PBCA nanoparticles are well known in the literature and for this study it was possible to produce particles with an average size of 153.55 +/- 33.14 nm. The preparation method to produce the nanoparticles was kept the same from the beginning through to the end due to the fact that a desirable particle size was achieved in our initial experiments. Sommerfeld et al, 1997 reported that factors such as the type of reaction vessel, amount of solution and monomer, size of the stirring bar, stirring speed and place and speed of adding the monomer can result in size differences of the nanoparticles (Sommerfeld et al, 1997). Such factors

could explain the size difference between the smallest and the largest nanoparticles produced in our laboratory.

After producing nanoparticles with a desirable particle size, the next step was to load 5-FU into the PBCA. Studies have shown that 5-FU may act as initiator of the polymerization of the cyanoacrylate monomer through either an anionic or zwitterionic mechanism depending on their structure and reaction conditions (Simeonova et al, 2003).

5-FU was incorporated as a drug model into PBCA nanoparticles. The size of empty nanoparticles and 5-FU nanoparticles were compared and the drug loading efficiency and 5-FU's cytotoxic effect on lung cancer cells were evaluated. Both nanoparticles and 5-FU nanoparticles were prepared by an emulsion polymerization method. The monomer was added to an HCl solution containing Dextran (polymerization medium) under constant mechanical stirring to polymerize the cyanoacrylate. 5–FU was dissolved in the polymerization medium at three different times:

a) Before the addition of the monomer
b) One hour after the start of the polymerization
c) Three hours after start of the polymerization.

These results showed that the time that 5-FU was added to the solution of nanoparticles did not have an impact on the drug loading of the PBCA nanoparticles. It also showed that only one hour of polymerization of the nanoparticles is enough to get 5-FU incorporated, encapsulated or attached into the nanoparticles.

All methods used had achieved similar results, except for the one in which diethylaminoethyl–dextran hydrochloride was used as a stabilizing agent instead of Dextran 70. DEAE–dextran is a polycationic derivate of dextran containing diethylaminoethyl groups coupled to the glucose residues by ether linkages (Douglas et al, 1986). 5-FU and diethylaminoethyl–dextran hydrochloride did not work in this method because both substances are cationic in an anionic medium. The cationic tertiary and quaternary amino groups from the DEAE-dextran gave the polymer a positive charge, producing cationic nanoparticles. Due to this reason, there was no attraction between 5-FU and the positive charged nanoparticles. Therefore it was assumed that there was no drug attached, encapsulated or covalently attached to the nanoparticles. This sample was only analyzed for drug entrapment efficiency.

The polymerization of the nanoparticles was conducted at a pH between 2 and 3. The reason for that is that OH^-, which results from the dissociation of water, initiates the polymerization of the nanoparticles. Consequently, the pH has to be acidic in order to prevent excessively rapid polymerization and enable the slow formation of nanoparticles (Kreuter, 1990). During polymerization Dextran 70 was used as a stabilizer. Furthermore, when the polymerization is too fast, it can lead to

the formation of agglomerates and consequently an increase in particle size (Puglisi et al, 1993). Most of the researchers allow a minimum time of at least two hours for polymerization. In our experiment we used a minimum time of four hours since literature suggests that a shorter time may lead to nanoparticles with a larger size.

Successful nanoparticles are the ones that have a high loading capacity and optimal release properties in order to decrease the amount of nanoparticles required for administration (Pison et al, 2006; Soppimath et al, 2000). Due to these two characteristics in the beginning of the project, it was assumed that a large amount of 5-FU was incorporated into the nanoparticles. Cell culture results were performed to analyze whether or not the drug was successfully loaded into the nanoparticles. The loading efficiency of 5-FU nanoparticles was high (89%) and good results were obtained with Confocal Microscopy when the uptake of 5-FU in lung cancer cells was investigated. Results from the confocal pictures showed the uptake of 5-FU nanoparticles in A-549 lung cancer cells. However results obtained from the cell culture indicated that there was no cytotoxic response between the 5-FU nanoparticles and lung cancer cells. These results were contradictory with the drug loading results cited earlier. The results have shown that polybutylcyanoacrylate nanoparticles were not a successful carrier particle for 5-FU. This might have happened due to the fact that 5-FU acted as the polymerization initiator and become covalently bound to the polymer. In this case 5-FU would not be released. Simeonova et al, 2004 showed that 5-FU was the initiator in polymerization of the nanoparticles and this fact leads us to believe that, in our experiments, no 5-FU was released. This could explain why there

was no drug release when 5-FU was tested using the lung cancer cells. According to Mccarron et al, 2000 5-FU is a hydrophilic compound and because of this, it is to be expected that loading from an aqueous polymerization phase into a hydrophobic particulate core is low (Mccarron et al, 2000). In this study it was also found that 5-FU had considerable problems and produced poor loading, particularly in alkylcyanoacrylate particles (Mccarron et al, 2000).

For future experiments it will be necessary to investigate the drug loading capacity of 5-FU to nanoparticles in more detail. This may include to try new methods of synthesis of 5-FU nanoparticles or using another type of polymer, for example, Eudragit ® (Lamprecht et al, 2003). There exists a large range of types of Eudragit with different properties. These properties are based on its structure. They are synthesized by copolymerization of methacrylic acid, methyl acrylate and methyl methacrylate (Lamprecht et al, 2003). Therefore the use of other analytical methods such as high performance liquid chromatography (HPLC), gas chromatography (CG) or GC–mass spectrometry may be a better alternative to the UV spectroscopy. Other alternatives would be to increase the concentration of 5-FU and the amount of polymer added, in order to increase the loading efficiency of 5-FU in the nanoparticles.

4.3 Development Carrier Particles

4.3.1 Development of Lactose Based Carrier

An aqueous solution containing 7% lactose was spray dried using the following parameters: inlet and outlet temperature of 140-160 °C and 110 °C respectively, aspirator 15 (out of 20), flow meter 800 NormL/hr and a feed rate of 2 mL/min. A higher inlet temperature was necessary because water was the only solvent that was being used in this formulation. The mass median aerodynamic diameter of the carrier particles was analyzed using the Andersen cascade impactor. Ten samples of the lactose carrier particles were analyzed. The results showed that the mean MMAD was 10 μm or larger. The fine particle fraction (FPF) was found to be 13.86 +/- 5.56 % (n=8). The emitted dose for powders made only of lactose was found to be 73.38 +/- 13%. The shape and the morphology of the lactose carrier particles were investigated using SEM. Figure 4.3 shows a lactose powder that was made without the presence of any other solvent or excipients. The image shows that most of the particles in this powder were spherical and had a smooth surface. In addition the particles were very small. Particles like this are heavier and have a higher density because they do not present any hollow matrix or a porous surface.

Figure 4.3 SEM image of typical lactose particles observed after spray drying of a 7% lactose solution.

4.3.2 Development of Effervescent Carriers

To produce inhalable effervescent powders, the first step was to establish an effervescent formulation. The basic formulation contained sodium carbonate, sodium bicarbonate, citric acid, ammonia and water. In order to prevent an effervescent reaction from happening before spray drying, the pH of the solution was increased using ammonium hydroxide in order to maintain a pH between 7.0 and 9.0. At this pH, the acid and carbonate react very slowly with each other. The ammonia evaporates in the spray drying process and the resulting powder contains citric acid and carbonate in the solid state. The resulting powder exhibits effervescent properties when it comes in contact with water or humidity, which includes moist air. The

effervescent effect of the carriers was visible when the carrier particles came in contact with water or humidity. The powder was stable under dry conditions.

The effervescent effect of the carrier particles was investigated using confocal microscopy. The confocal microscopy allows the visualization and characterization of the carrier particles not only on the surface, but also inside the carrier particles. The laser was adjusted in the green/red fluorescence mode and these colors were obtained from two separate channels. The carrier particles were labeled with a red color and the nanoparticles with a green color. To observe both carrier particles and nanoparticles, the final pictures were composed from overlaying the red fluorescence with the green fluorescence. The effervescent properties of the carrier particles were observed after the carrier particles were exposed to water, aqueous surfaces or moist air. Figure 4.4 shows carrier particles (before being exposed to humidity), which were approximately 15 μm in diameter. These were hollow particles with a spherical shape (Figure 4.4 A & B). The nanoparticles were distributed continuously throughout the hollow carrier particle matrix.

A) B)

Figure 4.4 Carrier particles containing nanoparticles (before being exposed to humidity).

A) Green nanoparticles are distributed throughout the hollow carrier particle matrix.

B) Red carrier matrix.

The effervescent imaging was prepared using the same method as the previous sample, but this one was exposed to humidity. With the humidity effect on the effervescent carrier particles, it was possible to immediately visualize the formation of bubbles in the immersion oil. Figure 4.5 shows the swollen and dissolved carrier particles after exposure to humid air (Figure 4.5 A, B, C). The matrix of the carrier particles dissolved (red channel) showing a green bubble of more than 30 μm filled with nanoparticles. This indicates that the nanoparticles were actively distributed throughout the gas bubble. When the effervescent powder was dispersed in water, small gas bubbles were visible immediately after dispersion.

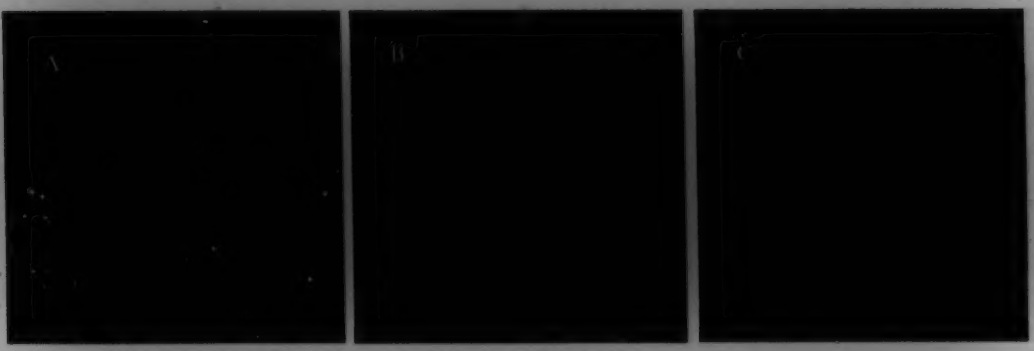


Figure 4.5 Confocal microscopy pictures of effervescent particles exposed to humidity.

A) Super imposed red and green channel showing gas bubbles of different diameters

B) Green channel showing the nanoparticles distributed throughout the gas bubble

C) Red channel showing the dissolved carrier matrix.

4.3.3 Discussion

The challenge of any powder for inhalation is to produce particles with a suitable size for pulmonary inhalation. The main issue for dry powder formulation is particle aggregation that consequently increases particle size (Bosquillon et al, 2001). In consideration of that, the most important aim of this work was to create a new type of carrier particle and to optimize its formulation. First, lactose carrier particles were produced and the results were compared to a new type of carrier particle. The new type of carrier particle had an active release mechanism. The objective of having particles with an active release mechanism is to have a faster release of drugs and/or nano-materials. The optimization of the carrier particle was necessary in order to achieve a required size distribution for deep lung deposition. Deep deposition requires

particles with an aerodynamic diameter below 5 μm (Bosquillon et al, 2001; Dailey et al, 2003; Lucas et al, 1999).

The lactose carrier particles were used as a basic type of carrier. Lactose was chosen as the carrier since it is the most common type of carrier particle and today, it is approved by the Food and Drug Administration (FDA) and other regulatory organizations for inhalation purposes (Bosquillon et al, 2001). Carrier particles can also be made of mannitol. Although lactose is the most common type of carrier particle, mannitol is an option to produce particles in order to prevent a possible Millard reaction (Kambo et al, 2006). A Millard reaction is the reaction between the reducing sugar and an amino group, for example an aminoacid.

The results obtained when lactose carrier particles were produced showed particles with a larger MMAD and a smaller FPF but with a high emitted dose. Particles with a larger MMAD and small FPF have only a very small chance of being deposited in the alveolar region of the lungs even if the value of emitted dose is high. *In vitro* results indicated that most of these particles would be deposited in the upper respiratory tract since more than 60% of the carrier particles were deposited in plates 0 and 1 of the impactor. One possible reason for the large particle size of the carrier particles is particle aggregation. Particle aggregation could have increased the aerodynamic diameter and consequently impeded deep lung deposition. A possible solution of this problem is to add substances such as lubricants (L-leucine), surfactants (SLS), emulsifiers like dipalmitoylphosphatidylcholine (DPPC) or

albumin (Bosquillon et al, 2004) or alcohols (ethanol) to the formulation. In order to overcome these issues, a new formulation was investigated.

Traditionally effervescent formulations contain two components: an acid and a base. In this project, a new system of carrier particles was developed that uses effervescent technology. The difference between the traditional system and ours is that we used ammonium hydroxide in our formulations. This technology is new and it was used for the first time. The effervescent reaction releases carbon dioxide. The phase transition from a solid to the gas phase increases the volume. This is used in tablets to increase tablet disintegration and drug dissolution (Karhu et al, 2000). This fact shows that the effervescent particles can help the drug dissolution of the carrier particles. In oral tablet formulations, effervescent technology uses a mixture of acids (e.g. citric acid) and carbonates. A typical ratio of carbonates that generally achieves a fast effervescent reaction and acceptable stability uses a mixture of 50% sodium carbonate and 50% sodium bicarbonate (Rau, 2001). However, sodium bicarbonate decomposes at temperatures above 50 ºC and for this reason it is not recommend to be used for spray drying procedures, which uses temperatures that will exceed this value.

The effervescent reaction might be used to create airborne nano- or micro-droplets containing molecules or active principles in nano- or micrometer scales. The airborne droplets might increase the rate of release and also increase the dissolution of the material that is being used. These properties can be used to improve the drug delivery systems that are now available or to create new ones. However

more studies are required in order to get more specific results of such an approach and to investigate the mechanism responsible for enhancing the dissolution. Therefore not all drugs and nanoparticles may be used for this method since a stability problem may occur due to the pH requirement of approximately 8. There exists a large range of substances or active principles that could be incorporated into the effervescent carrier particles. Some examples are amino acids, proteins, peptides, polypeptides, diagnostics, imaging agents, enzymes, radiopharmaceuticals, nucleotides, nutrition, vitamins, hormones, immunomodulating agents, cytokines, anti bodies, anti bacterial agents, anti viral agents, mucolytic agents, bioadhesive agents, vaccines, surfactants, viscosity inducing agents, viscosity reducing agents, metal and metallic compound, natural and herbal ingredients, chelating, binding and/or absorbing agents.

4.4 Spray Dryer Parameters and Formulation Components

In order to optimize the synthesis of the carrier particles and to achieve a suitable formulation for deep lung deposition, different spray drying parameters and formulation ingredients were investigated. It was previously reported that the powder composition and solution properties greatly affected particle characteristics (Bosquillon et al, 2001). The effect of these parameters on particle size, shape, mass median aerodynamic diameter, fine particle fraction, and morphology were investigated.

4.4.1 Spray Drying Parameters

A spray dryer can be used to produce dry powders from solutions, suspension or emulsion. This technique allows us to have greater control over particle size, morphology and powder density (Rabbani and Seville, 2005). The properties of the final powder depend on the inlet and outlet temperatures, aspirator, feed properties, nozzle size and airflow.

4.4.1.1 Inlet and Outlet Temperatures

Three different inlet temperatures, 93 °C, 123 °C and 165 °C were investigated in order to evaluate their effect on shape, aerodynamic diameter and morphology of the carrier particles. Other parameters were kept the same. In this way, it was possible to analyse only the effect of temperature on the carrier particles.

For inlet temperatures at 93 °C, 123 °C and 165 °C the MMAD was 5.22, 3.85 and 8.3 μm respectively. The most desirable MMAD for deep lung deposition was 3.85 μm when an inlet temperature of 123 °C was used.

Figure 4.6 A, B & C shows the difference in shape and morphology when the temperature increases. The scanning electron microscopy indicated that when temperatures increase, the particle morphology changes from a sponge-like shape to a more spherical shape. It also decreases particle size while tending to increase particle

density. Figure A shows particles that are very porous and that have a sponge like shape whereas Figure B presents particles with irregular and spherical shapes and a porous morphology. Finally, Figure C shows particles with a more spherical shape and a smooth surface but a larger variety of particle sizes was observed.

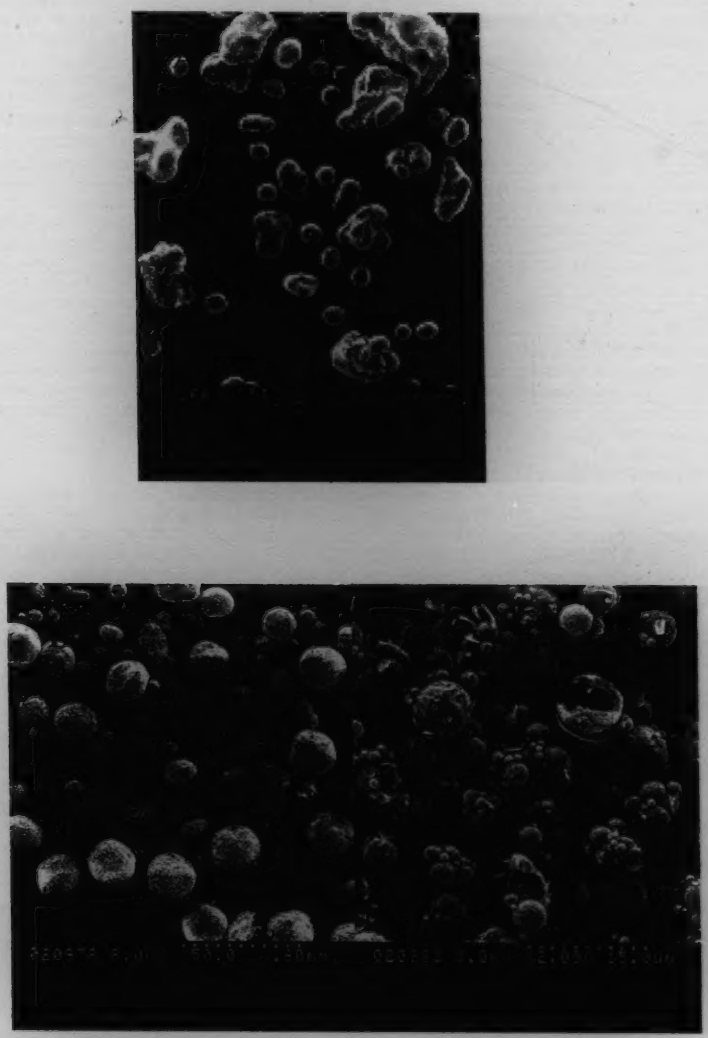


Figure 4.6 SEM image of effervescent powders spray dried at different inlet temperatures.

A) 93 °C B) 123 °C C) 165 °C

4.4.1.2. Airflow Rate

The spray flow rate is the amount of compressed air needed to disperse the solution. A gas other than compressed air can be used. The airflow is a regulator of moisture in the final product as well as the temperature. To investigate the effect of the atomization on the carrier particles, flow rates of 600 NormL/hr and 800 NormL/hr were used and the MMAD and FPF were calculated. The MMAD values found were 10 μm for a flow rate of 600 NormL/hr and a 6.47 μm for 800 NormL/hr. The FPF were 5.9% and 11.29% respectively. Our best results were obtained when a flow rate of 800 NormL/hr was used. The results showed that using a higher flow rate, smaller carrier particles were produced.

The experiments are shown in Figure 4.7 A & B. These figures show the distribution of the carrier particles through the plates from the impactor, using flow rates at 600 NormL/hr and 800 NormL/hr. Graph A has 88.88% of the carrier particles distributed in plate 0 and only 10.41% of the particles under were 5.6 μm. The results showed that the MMAD was larger than 10 μm. Graph B showed a better distribution of the powder. 55.69% was in plate 0, 15.18% in plate 1, 9.49% in plate 2, 14.56% in plate 3 and 5.07 % in plate 4. The figures below illustrate the particle distribution of an effervescent formulation containing lactose (4g), sodium carbonate (2.5g), sodium bicarbonate (2.5g) and citric acid (4g) when different flow rates were used with the following spray drying parameters: Pump 2 mL/min, Aspirator 8 (out of 20); Inlet temperature 64 °C and Outlet temperature 50 °C.

Figure 4.7 A) 600 NormL/hr

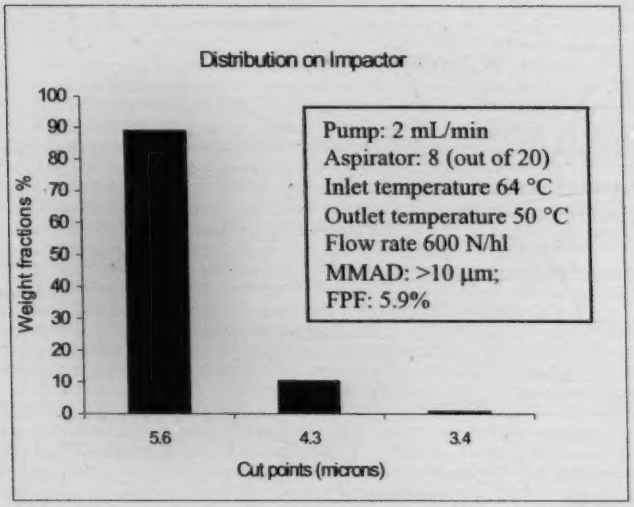


Figure 4.7 B) 800 NormL/hr

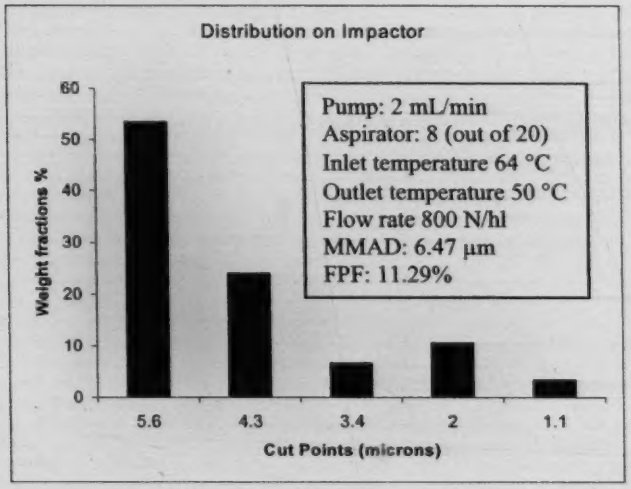


Figure 4.7 Distribution of the effervescent powders on the cascade impactor.

Figure A shows the distribution of the powders on the Andersen Cascade impactor when a flow rate of 600N/hl was applied. Figure B show the distribution of the powders when a flow rate of 800 N/hl was used.

4.4.1.3 Pump

The peristaltic pump feeds the spray solution to the nozzle. The pump flow rate may affect the characteristics of the final product. Pump settings used were 2, 3 and 5 from a dial setting (range 0-20). Results using different pump settings did not show any significant difference between the powders. However it was observed that when a pump setting of 5 was used, the final powder was not dried enough. For this reason, the experiments were conducted using a pump setting of 3, which represents 2 mL per minute.

4.4.1.4 Aspirator Flow Rate (quantity of air)

The drying air is sucked or blown through the device by the aspirator motor creating under pressure conditions. By regulating the aspirator speed, the amount of heated drying air can be increased or decreased (BŰCHI Labortechnick AG–Training Papers Spray Drying). The spray dryer solutions were tested using an aspirator setting between 7 and 15 (out of 20) and the results were investigated. To evaluate the aspirator results, the MMAD of the spray dryer samples were used in order to find the best setting. The best results were achieved using an aspirator setting of 10 and 15 since smaller MMAD (below 5 μm) were achieved. To produce the effervescent powders an aspirator setting of 15 was maintained.

In summary, Table 4.3 shows the results obtained in our spray drying experiments. It shows the main parameters used and their results. According to the table, the smallest MMAD and the highest FPF were achieved using the following parameters: inlet temperature of about 120-125 °C, atomization air of 800 NormL/hr, pump 2 ml/min and an aspirator setting of 10 and 15 (out of 20). When the powders were spray dried and then followed by a jet milling process, the results demonstrated some improvement compared to spray drying alone. Although results were positive for spray dryer and jet mill combined, time and cost of production would be high compared to using the spray dryer alone. This makes spray drying and jet milling as combined manufacturing steps not a desirable method for large scales of production.

Aspirator (out of 20)	Atomization air	Inlet temperature	MMAD (μm)	FPF (%)
15	800 NormL/h	170 °C	10	8.3
15	800 NormL/h	170 °C	10	14.45
10	800 NormL/h	123 °C	6.52	23
10 *	800 NormL/h	123 °C	10	8.3
15	600 NormL/h	125 °C	10	5.9
15	800 NormL/h	125 °C	6.47	11.29
15 **	800 NormL/h	125 °C	6.70	35.7

* Sample collected from the cyclone ** Spray drying and jet milling

Table 4.3 Process parameters and powder properties

The powders generated by spray drying resulted in particles with a large range of geometrical sizes. Particles presented a geometrical size between 0.5 and 15 μm.

Some of these particles appeared to be smooth while others were porous. The geometric size alone is not capable of determining the MMAD.

4.4.6 Formulation Composition

In this part of the project, the influence of formulation components on shape, morphology, aerodynamic diameter (MMAD), fine particle fraction (FPF), geometrical standard deviation (GSD) and emitted dose were investigated. Some excipients that are cited in the literature and are well known were tested (Bosquillon et al, 2001, Gliński et al, 2000; Rabbani and Seville, 2005). Most of these ingredients have been used to produce carrier particles or are used as excipients in effervescent tablets. Ingredients such as ethanol, polysorbate 80, L-leucine and PEG 6000 were added to the basic formulation to improve the particle size and to achieve an appropriate MMAD. The surfactants and lubricants were chosen from a selection of excipients considered suitable for inhalation (Bosquillon et al, 2001; Tsapis et al, 2002; Vanbever et al, 1999) or are proved to be safe for human use. In addition, the effect of different concentrations of lactose on the carrier particles was tested.

The spray dryer parameters were kept the same for all formulations tested. The parameters applied were: inlet temperature of about 120-125 °C, air flow of 800 NormL/hr, pump setting of 3 (2 ml/min) and an aspirator setting of 15 (out of 20).

The components and the concentration tested are shown in Table 4.4.

	Ingredients used	Concentration tested (%) or mL used
Carbonates	Sodium Carbonate	0.75 % – 1.5%
Acid	Citric Acid	1.2%
	Ammonia	10-30 mL
Lubricants	L-leucine	0.8 -1%
	Polyethylene glycol 6000	0.8 -1%
Alcohols	Ethanol	10- 30%
Surfactants	Polysorbate 80, Sodium Lauryl Sulfate	1%

Table 4.4 Formulation composition and concentration tested

4.4.2.1. Polysorbate 80 and Sodium Lauryl Sulfate

Polysorbate 80 and sodium lauryl sulfate (SLS) are surfactants. Polysorbate 80 is a hydrophilic nonionic surfactant (Zhang et al, 2003) and SLS is an anionic surfactant. Surfactants are agents that reduce the surface tension of a liquid and also lower the interfacial tension between two liquids. They are usually organic compounds that contain a hydrophobic group and a hydrophilic group. The addition of these ingredients did not show any improvement with respect to particle size but resulted in a smaller amount of powder being lost in the dry chamber. Furthermore, they did not affect the overall morphology of the effervescent powders. When polysorbate 80 was used, the MMAD was 10 μm (n=3) and FPF was 12.50% (n=1).

When SLS was used, the MMAD of the particles was larger than 10 μm and, in this case, the FPF was not investigated.

4.4.2.2 Ethanol

Ethanol was added to the formulation with the objective of producing larger porous particles with a lower density. There are many references in literature which use ethanol to produce such particles (Edwards et al, 1998; Tsapis et al, 2002). Our results showed that when ethanol was used, it did not improve the particle size or the morphology of the carrier particles. The MMAD was still approximately 8.5 μm and the FPF was 17.87 %. These results are not suitable for deep lung deposition. This might be due to the relatively low ethanol concentrations used in our formulations which were between 10-30 % v/v. These concentrations appear to have no effect on the porosity of the carrier particles.

4.4.2.3 Different Concentrations of Lactose

Formulations containing 1.2% lactose, 2.4% lactose, 3.5% lactose and 10 % lactose were tested. Results showed that different amounts of lactose had a large impact on the size and morphology of the carrier particles. It is clear that the concentration of lactose affects the properties of the final powder. Figure 4.8 A, B, C & D show a sequence of SEM pictures with increasing concentrations of lactose. Figure 4.8 shows that increasing the amount of lactose led to smaller and denser

particles and also produced particles with more spherical shape. These results are in agreement with results reported by Vanbever et al, 1999 (Vanbever et al, 1999). In addition to the changes in shape and morphology of the carrier particles, the MMAD also increased with the addition of lactose.

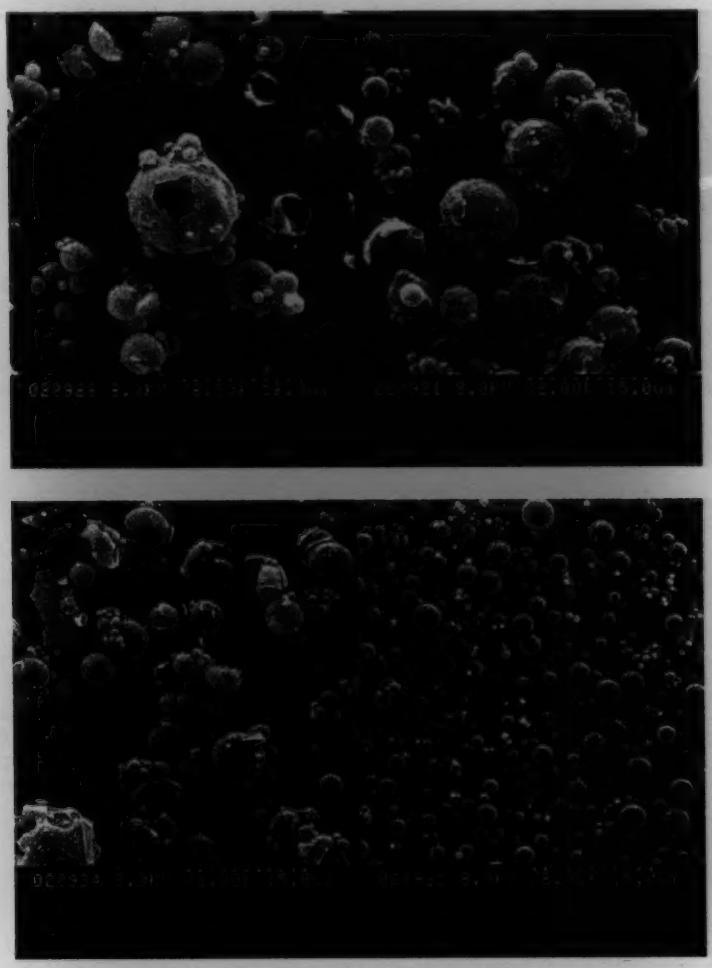


Figure 4.8 SEM image of spray dried lactose samples using different concentrations of lactose.

A) 1.2 % B) 2.4 % C) 3.5 % D) 10 %

4.4.2.4. L-leucine and Polyethylene Glycol 6000

A large improvement in particle size and MMAD was observed when 5 mL each of a 2.5 % (w/v) solution of L-leucine and 2.5% (w/v) solution of PEG 6000 were added to the formulation. For these effervescent carrier particles, the average MMAD was 2.17-μm +/- 0.42, FPF was 46.47 % +/- 15 and the GSD was 2.00 +/- 0.06. The GSD values characterized the polydispersity nature of the distribution of the aerosolized particles of this formulation. Using L-leucine and PEG 6000 in the effervescent formulation, it was possible to obtain inhalable particles as indicated by the SEM pictures in Figure 4.9. These particles also show a more irregular morphology when compared to the highly spherical lactose carrier particles. L-leucine allowed the preparation of powders with better aerolization properties and particles with low density (Lucas et al, 1999). Li et al, 2003 showed that L-leucine is effective in eliminating the aggregation of peptide and protein dried powders (Li et al, 2003). PEG 6000 produced particles with a more aspirated and irregular surface as described by Corrigan et al, 2002 and Mu et al, 2005 (Corrigan et al, 2002; Mu et al, 2005).

Figure 4.9 shows a scanning electronic microscopy image of effervescent carrier particles prepared with L-leucine and PEG 6000.

Figure 4.9 SEM image of effervescent carrier particles prepared with L-leucine and PEG 6000.

4.4.3 Emitted Dose:

The emitted dose was calculated for lactose and effervescent powder and the difference between the two were compared. The emitted dose for powders made of lactose alone was found to be 73.38 +/- 13% and for powders containing the L-leucine/PEG 6000 effervescent formulation were 68.55 +/- 23.90%. There is no statistical difference between the emitted doses of these powders.

Table 4.5 summarizes the results of the tested formulations. It shows the results for MMAD, FPF and GSD. It shows that the only formulation suitable for deep lung deposition is the one that contains both L-leucine and PEG 6000. This formulation presented a smaller MMAD, a higher FPF and a GSD of 2.00 +/- 0.06 when compared to the other formulations.

Powder	MMAD (μm)	FPF (%)	GSD
Blank lactose (7%)	10	13. 86 % +/- 5.56	-
Ethanol 30%	8.5 +/- 1.8	17.87 +/- 4	-
Lactose 10%	10	17.60 +/- 3.5	-
Tween 80	10	12.50 +/- 2	-
L-leucine and PEG 6000	2.17 +/- 0.42	46.47 % +/- 15	2.00 +/- 0.06

Table 4.5 Formulations used for spray drying and their size distribution.

4.4.4 Water Content:

The loss of drying was calculated as follows:

$$\text{Eq (4.1)} \quad \frac{\text{Weight of sample before drying} - \text{weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

Figure 4.10 shows that there is no difference in the moisture content between samples before and after being kept in a vacuum oven for 48 hours. The samples were kept in a desiccator immediately after being spray dried, and in that way they were moisture free.

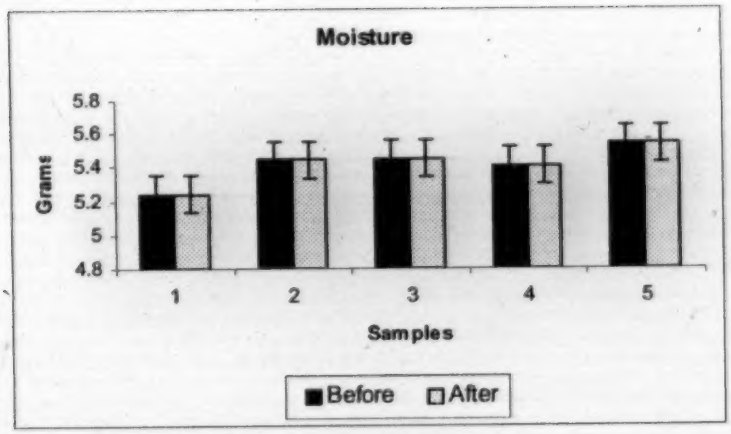


Figure 4.10 Moisture content of the samples

4.4.5 Discussion

4.4.5.1 Spray Drying Parameters

The optimization of the spray drying conditions was performed to design a final product with desired particle size. Formulation and process parameters are important to produce carrier particles with an appropriate MMAD. Results showed that inlet temperature and air flow had the greatest effect on the aerodynamic particle size while pump and aspirator settings had only little impact on powder properties. Besides an effect on the MMAD of the carrier particles, the inlet temperature also had an impact on the shape and morphology of the carrier particles. The best particle shape and morphology was achieved using a temperature of 123 C° that produced particles with irregular shapes and a porous morphology. According to Chew at al, 2002, the irregular shape and surface of the carrier particles can help prevent them

from being in close contact with each other, resulting in reduced cohesion and thus better dispersion. The reason for that is that particle cohesion depends on surface contact. For example, irregular surfaces would translate to lower interparticulate cohesion and easier dispersion of the carrier particles (Chew at al, 2002). Edwards et al, 1997, investigated the effect of the porous particles. Edwards and his colleagues have shown that porous particles improve the aerolization of the powders from the inhalers and improve deep lung deposition. This occurred due to reduced particle mass and therefore reduced chance of the particles being taken up by macrophages in the lung (Edwards et al, 1997).

Appropriate spray drying conditions may be selected for producing carrier particles so that the biological activity of the drug can be maintained. For example, if a drug is known to decompose at higher temperatures, lower temperatures may be used to prepare the particles. To be able to obtain inhalable particles, it is fundamental to adjust the spray dryer parameters.

An advantage of producing inhalable powders via spray drying is that one has control over the spray dryer and is able to produce powder particles with different MMADs. This makes it possible to deliver a powder to different areas of interest within the respiratory tract including the bronchial region or nasal cavity (Finlay, 2002). Aerosol powders can be produced to treat different diseases acting systemically (e.g. asthma) or locally (e.g. lung cancer) within the respiratory tract. An additional advantage of the spray drying method is that it can be applicable to both

heat-resistant and heat-sensitive drugs as well as to both water-soluble and water-insoluble drugs (Mu et al, 2005).

4.4.5.2 Formulation Composition

In this part of our work, different powder compositions were produced in order to develop and to optimize an effervescent aerosol carrier particle formulation. Our results showed that different amounts of lactose had a large impact on size and morphology of carrier particles. When the lactose fraction was increased, it led to smaller and denser particles. It also produced particles with a more spherical shape. These results are in agreement with results reported by Vanbever et al., 1999. These results and Figure 4.3 show clearly that the carrier particles containing only lactose in their formulations were not desirable for lung deposition. However lactose is fundamental to produce the carrier particles. It simply needs the inclusion of ingredients that modify the bulk properties of the formulation and help to achieve better aerolization properties and to improve particle deaggregation.

Ethanol was added to the formulation with the aim of producing larger porous particles with a lower density. Our results showed that when ethanol was used, it did not improve the particle size or the morphology of the carrier particles. The MMAD was still approximately 8.5 μm and was not suitable for alveolar deposition. This might be due to the relatively low ethanol concentrations used which was between 10-30 % v/v. Other studies have reported using up to 70% of the total volume of

ethanol to produce porous particles (Tsapis et al, 2002). However we could not use a higher amount of ethanol because of safety reasons. Polysorbate 80 did not show any improvement in particle size when compared to the lactose formulation.

The most pronounced effect on particle size occurred with the addition of L-leucine and PEG 6000 which improved the aerodynamic characteristics of the powder particles. The *in vitro* results indicated that these particles were suitable for deposition throughout the lungs. Our result for the MMAD is almost equal to those reported by Bosquillon et al, 2001 (Bosquillon et al, 2001).

Powders produced by spray drying are usually more cohesive, leading to an inadequate dispersion during aerolization. It has been demonstrated that incorporation of some lubricants, such as L-leucine and some types of PEG into the formulation can generate powders with better aerolization properties (Corrigan et al., 2002; Gilani et al., 2004; Glinski et al, 2000). This was confirmed in our results.

L-leucine is one of the most hydrophobic amino acids and is an analogue of isobutanol or isobutanoic acid. This can be one of the reasons that when L-leucine was added to a water solution, it caused a fast decrease in surface tension (Glinski et al, 2000). These results are also in agreement with other studies (Bosquillon et al, 2001; Li et al, 2003; Otsuka et al, 2001). In addition, the hydrophobic properties of L-leucine may allow the preparation of powders with better aerolization properties because it decreases the particle aggregation (Li et al, 2003). L-leucine is stable at

high temperatures and this makes L-leucine a good excipient to be used in any spray drying formulation. Furthermore, spray drying of powders containing L-leucine resulted in spherical hollow particles. The small tiles of L-leucine yield good lubrication and prevent adhesion of the carrier particles.

Corrigan et al, 2002, Gianni et al, 2004 and Mu et al, 2005 investigated the use of PEG in their formulations. They found that polyethylene glycol had a major impact on the size and morphology of carrier particles. Presence of PEG 6000 changed the surface texture of the carrier particles from a smooth surface to a more aspirated surface. Mu et al, 2005 also reported that a high ratio of PEG prevented well-defined particles but tended to increase irregular clumping. However, this might be due to the insolubility of PEG in an organic solvent (Mu et al, 2005). Similar effects were observed in our study using the effervescent formulation. Polyethylene glycol may influence the crystalline and polymorphic form of spray-dried lactose and, presumably, of incorporated drugs (Otsuka et al, 2001; Gliński, et al, 2000). Several authors have demonstrated that PEG has been used in fast release preparations (Asada et al, 2004; Lo and Law, 1996). The addition of PEG to the effervescent formulation might also help the dispersion of the drugs and nanoparticles.

Generally, when the carrier particles have a large median particle size, the FPF is smaller. This was confirmed by our results. When an MMAD between 8 μm and 10 μm was obtained, the FPF varied between 13 and 17%. When the MMAD was approximately 3 μm, the FPF increased to 46.47 % +/- 15. Therefore, if an MMAD is

larger, less fine particles are present. However, according to Chew and Chan, 2002, when the particle size is too fine, the powders become very cohesive and difficult to disperse. This will also result in a small FPF. In order to solve this problem and to increase the dispersion of the powders from the impactor, airflow of 60 l/min was used. Using this flow rate, it was possible to obtain carrier particles with an appropriate MMAD and an acceptable FPF distributed into the plates of the impactor.

Another alternative for producing particles with higher FPF is to produce particles with an irregular shape and morphology also known as wrinkled particles as shown in Figure 4.9. As demonstrated by Chew et al, 2002, dispersion of wrinkled particles produced much higher FPFs than smooth ones. This was confirmed by the role played by the surface morphology of the carrier particles.

Results reported for the emitted dose are satisfactory for both the dry powder formulation containing only lactose and the dry powder L-leucine/PEG 6000 formulation containing effervescent carrier particles. According to Labiris and Dolovich, 2003, using conventional devices, only 12-40% of the emitted dose is delivered to the lungs and around 20-25% of the drug is retained within the device or the inhaler (Labiris and Dolovich, 2003). The effervescent powders with an emitted dose of 73.38 +/- 13% and 46.47 % +/- 15 were delivered to the deep areas of the lungs according to *in vitro* results. These values are larger compared to the values cited by Labiris and Dolovich (Labiris and Dolovich, 2003). The composition and the aerolization properties of our powder might have improved the emitted dose and FPF

of the carrier particles. However, the emitted dose achieved for the lactose carrier particles mostly contained powder particles which were too large for deep lung deposition. This powder was mainly deposited on plate zero of the cascade impactor while most of the effervescent powder was deposited on plates 2, 3 and 4. These were shown by the FPF of 13.86 +/- 5.56% for the lactose formulation and 46.46 +/-15% for the effervescent formulation. The percentage of the emitted dose deposited in the lungs is dependent on the powder dispersibility which is controlled by interparticle cohesive forces (Steckel et al, 2004). Producing carrier particles with an adequate shape and morphology can increase the emitted dose delivery to the lungs.

In summary, pulmonary formulations must have a small MMAD and high FPF in order to decrease central/tracheobronchial deposition and bypass the effects of mucociliary clearance (Cook et al, 2005). MMAD and FPF are the most important factors in the aerodynamic behavior of powder aerosols. However other factors are also fundamental and deserve attention as well in order to produce a desirable formulation. The factors are geometric particle size, particle density, powder composition, powder crystallinity, surface properties and powder cohesiveness. Therefore, successful development of carrier particles or therapeutics for administration by the pulmonary route requires an understanding of the pulmonary barriers, aerosol formulation and device to achieve optimal delivery. The type of excipients used and spray dryer parameters greatly influenced the final product. L-leucine and PEG 6000 in the formulation appeared necessary for the achievement

of optimal aerosolization properties. In addition, as the right formulation is achieved, these formulations should be tested *in vivo* for local or systemic drug delivery.

4.5 Lactose carrier particles and PBCA nanoparticles

Polybutylcyanoacrylate nanoparticles were spray dried in an aqueous solution containing 7% lactose and in a solution containing 7% lactose, L-leucine and PEG. The parameters were the same as used to produce empty lactose carrier particles. The particle diameter of the nanoparticles was measured before and after the spray drying. The size before spray drying was 126.17 +/- 20.20 and the size after spray drying was 259.00 +/- 52.70. A t-test was used to compare the sizes of the nanoparticles before and after spray drying at $P < 0.05$. The nanoparticles differed significantly in their size after being spray dried. The MMAD found for the lactose carrier particles containing only lactose was 10 μm. However for the formulation containing lactose, PEG and L-leucine, the size of the nanoparticles before spray drying was 2.47 +/- 13.4 nm and 225 +/- 11.17 nm afterwards. Here, a t-test did not indicate a statistical difference between the nanoparticles before and after the spray drying process.

Figure 4.11 shows the size of the nanoparticles before and after spray drying using lactose carrier particles.

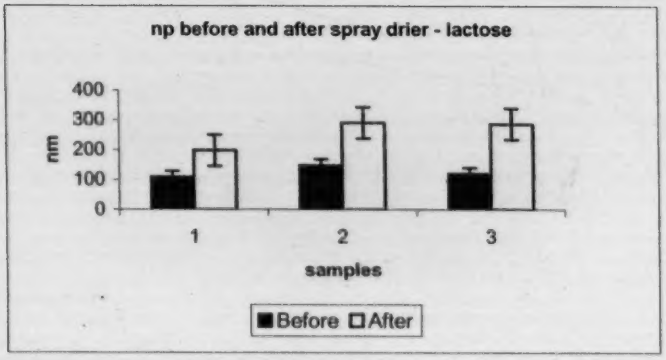


Figure 4.11 Nanoparticles before and after spray dried using lactose preparations

4.5.1 Incorporation of Nanoparticles into Effervescent Carriers

Polybutylcyanoacrylate nanoparticles were spray dried in an aqueous solution containing the effervescent formulation and the effervescent formulation containing L-leucine and PEG. The particle diameter of the nanoparticles was measured before and after spray drying. A t-test was performed to compare the sizes of the samples before and after spray drying. For the effervescent particles the results were 244 +/- 26.8 nm and 252+/-29 nm before and afterwards respectively. Using the effervescent preparations containing L-leucine and PEG 6000, the size before spray drying was 149.9 +/- 26.46 nm and the size after spray drying was 176.83 +/-15.45 nm. For the last two formulations, a t-test did not indicate a statistical difference between the nanoparticles before and after the spray drying process. The effervescent carrier particles presented a geometric size of 5 μm. The geometric size was measured using the confocal microscopy. Figure 4.12 shows the size of the nanoparticles before and after spray drying using effervescent carrier particles.

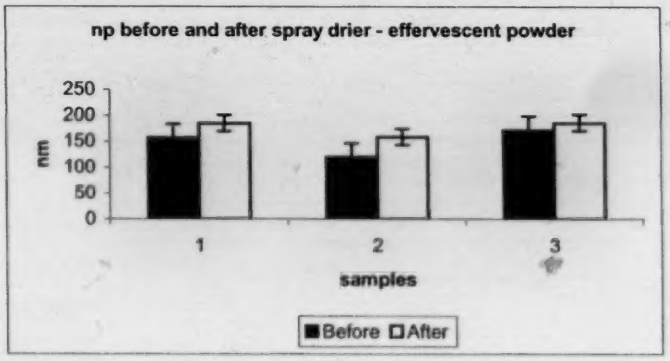


Figure 4.12 Nanoparticles before and after spray drying using effervescent preparations

4.5.2 Discussion

Spray drying is a well-known method used to prepare regular and/or larger porous carrier particles. Such carrier particles can contain nanoparticles. Edwards et al, 1998 demonstrated the advantages of the large and porous aerosol particles containing nanoparticles. These particles possess low mass density and a larger geometric size. According to Tsapis et al, 2002 these particles are characterized by geometric sizes larger than 5 μm and mass densities around $0.1 g/cm^3$ or less. Larger and porous particles have some advantages such as: 1- decreased tendency to aggregate since larger particles aggregate less 2- more aerolization efficiency compared to conventional inhaled therapeutic aerosols particles 3- the potential for avoidance of alveolar macrophages clearance. All these factors allow drug release for longer periods of time with more efficiency and finally, improved powder flow and dispersion (Edwards et al, 1998; Hardy and Chadwick, 2000; Vanbever et al, 1999). The effervescent carrier particles produced in our lab also presented some of the

characteristics cited above. The carrier particles presented a geometric diameter of 5 μm and a MMAD below 5 μm. These values indicate that the density of the carrier particles is low. Also the carrier particles possess a porous morphology as shown in Figure 4.8 A. The presence of L-leucine and PEG 6000 in the formulation improved the aerolization properties of the lactose and the effervescent carrier particles. In addition to these characteristics, the effervescent carrier particles were able to release nanoparticles through an effervescent mechanism. The difference between the carrier particles containing nanoparticles produced by Tsapis et al, 2002 and the effervescent carrier particles is in the formulation composition and the spray drying parameters. While Tsapis used as a solvent 70% (total volume) ethanol, we used only water. Another difference is the low outlet temperature applied by Tsapis while, in our study, the effervescent particles required a higher temperature.

According to Tsapis et al, 2002 dense dried particles occur when the drying of the spray droplet is slow and consequently the nanoparticles have sufficient time to redistribute by diffusion throughout the evaporating droplet. An ideal carrier particle containing nanoparticles require a quick drying of the droplet and, in that way, the nanoparticles accumulate near the drying front of the droplet (Tsapis et al, 2002).

Figure 4.13 shows a spray dried effervescent particle with nanoparticles.

Figure 4.13 SEM image of a spray dried effervescent particle with PBCA nanoparticles

Our results showed that spray drying is an effective method in producing powders with an appropriate MMAD for lung deposition as well as to keep the nanoparticles with narrow particle size distribution. It also showed that nanoparticles formulations are easier to incorporate into the effervescent carrier particles without modifying their size or the size of the carriers.

The formulations containing effervescent release mechanisms and the lactose formulations that contained L-leucine and PEG 6000 were able to release nanoparticles with less agglomeration compared to the carrier particles made only of lactose which lack an active release. The mean nanoparticle size did not significantly change upon release when the nanoparticles were incorporated into an effervescent formulation. However, the mean particle size significantly increased upon release when only lactose was the carrier particle matrix. The results showed that both the

effervescent formulation and the choice of excipients had a major effect on the release of nanoparticles. Therefore the effervescent reaction appears to improve the dispersion of the nanoparticles from the carrier particle. This might be due to the effervescent reaction of the carrier particles which generated forces that helped the nanoparticles disperse more efficiently and avoid particle aggregation. L-leucine and PEG 6000 also decreased the tendency of the nanoparticles to agglomerate as indicated by the particle size differences between freshly synthesized nanoparticles and re-dispersed spray dried particles. Sham et al., 2004 conducted a study using lactose carrier particles containing nanoparticles. In the cited study, it was found that some clusters of nanoparticles were observed in the carrier particles which increased the nanoparticle size after spray drying. Our results compared to this study, showed an improvement when using the effervescent carrier particles compared to the lactose carriers.

There are other successful studies aimed at lung delivery, where nanoparticles or drugs were incorporated into microspheres or other types of carriers. A study conducted by Grenha et al, 2005 demonstrated that protein-loaded nanoparticles can be incorporated in microspheres using spray drying and the resulting powder presented suitable characteristics for lung delivery. The prepared dry powder contained protein-loaded nanoparticles using typical aerosol excipients. Roa et al, 2004 have shown that nanoparticles loaded with cytotoxic agents when carried by aerolized cluster bombs are able to kill cancer cells efficiently. They conducted a study using doxorubicin alone and doxorubicin loaded nanoparticles and assessed

them by two different lung cancer cell lines. The results were observed using confocal microscopy that shows the uptake of nanoparticles into the cytoplasm. Additionally, it was observed that doxorubicin bound to nanoparticles could kill cells more effectively than doxorubicin alone (Roa et al, 2004).

4.6- Comparisons of Drug Release from Effervescent and Conventional Carriers

Ciprofloxacin drug release from conventional lactose particles was compared with the effervescent formulation. The results showed that the effervescent carrier particles released 56 +/- 8 % ciprofloxacin into solution compared with 32 +/- 3 % when lactose particles were used, which is a significant difference (t-test, $P<0.05$). The remaining drug was visual as precipitate before filtering the solution. A ciprofloxacin formulation was developed with adequate ciprofloxacin loading and release properties.

Results from the cascade impactor suggest that ciprofloxacin would be deposited in different regions of the respiratory tract but predominantly in the alveolar region since most of the particles were in the size range of 4.9 +/- 1μm. Figure 4.14 shows the amount of three samples of encapsulated ciprofloxacin found at each stage of the impactor. The results showed that there was a large amount of the drug located in the inhaler. However there were also large amounts of ciprofloxacin on plates 2, 3 and 4. The ciprofloxacin found on these plates varied in size from 1.1 to 3.4 μm in

aerodynamic diameter. Particles in this size range are suitable for deep lung deposition.

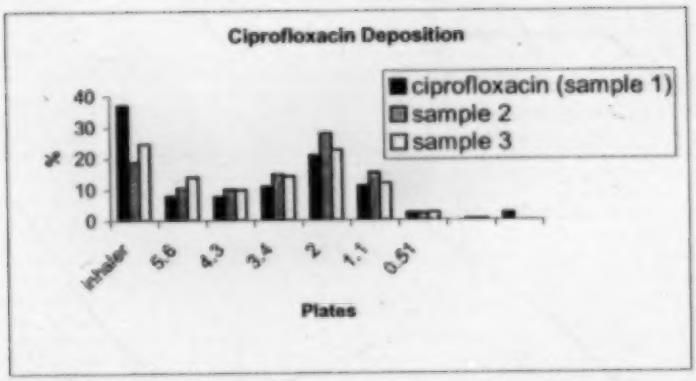


Figure 4.14 Distribution of ciprofloxacin on the impactor

The loading amount of ciprofloxacin in the carrier particles after spray drying was calculated as 23 μg/mg powder (S.D. = 3.2 μg/ mg, n=3). This result is in accordance with results reported by Sweeney et al., 2005. In his study, freeze spray-dried liposomal ciprofloxacin powder was investigated. The results showed that the liposomal ciprofloxacin powders were able to incorporated 20.6 μg/ mg (S.D. = 5.6 μg/ mg, n=5). Our results are slightly higher than the one found by Sweeney et al., 2005. This may have happened because liposome formulations may contain certain components (e.g. surfactants, concentration of lipoplexes) that can decrease the amount of ciprofloxacin encapsulated.

4.6.1 Discussion

Ciprofloxacin in this study served as a drug model for lactose and effervescent particles and was used in order to evaluate the effect of an active drug release from the carrier particles compared to passive release via dissolution. Ciprofloxacin was selected as the drug model because it is widely used as a broad-spectrum antibiotic to treat respiratory infections and is also used in patients with cystic fibrosis.

The results showed that effervescent carrier particles contained L-leucine and PEG 6000 and its formulation released a higher amount of ciprofloxacin compared with lactose carrier particles. It was assumed that the effervescent carrier particles were able to increase the drug dissolution since the dissolution extent was increased. Literature suggests that effervescent tablets have a faster release compared to conventional tablets and it might have the same effect for aerosol powders. Rygnestad et al, 2000, reported that effervescent paracetamol tablets were absorbed significantly faster compared to conventional tablets (Corrigan et al, 2002). Dosage form disintegration and drug dissolution are typically increased when effervescent formulations are used. Unfortunately, currently there is no method to determine the rate of dissolution for inhalable effervescent powders. An experiment was made in order to evaluate the effervescent effect. The cascade impactor plates were put into a dissolution apparatus. However, the effervescent reaction happened too fast and there was no difference observed in the drug release over time. The drug release was nearly instantaneous.

The amount of encapsulated ciprofloxacin found, in our study, at the stages of the impactor and also in the inhaler was expected. It is very difficult to produce inhalable powders where all the particles have the same particle size. Our impactor measurements indicated very promising results since approximately 57% of the powders were found at particle sizes varying from 1.1 to 3.4 μm. Therefore it is important to have a good inhaler that will deagglomerate the powder for inhalation (Voss and Finlay, 2002).

Conley et al, 1997 described the development of a liposomal formulation for the encapsulation of ciprofloxacin. It was found that the retention of the drug in the lungs of mice (via intranasal route) was improved significantly from a half-life of 1-2 hours to 8-10 hours. It was also found that liposome-encapsulated ciprofloxacin was significantly more effective than free ciprofloxacin in treatment of bacterial infections of the respiratory tract in mice. Similar results between the loading amount of ciprofloxacin effervescent carrier particles and investigated freeze spray-dried liposomal ciprofloxacin powder, cited above, were observed. The use of the effervescent powder is a possible alternative to the use of liposomes. The methods of preparation of the effervescent carrier particles are easier, compared to those of the liposomes. Therefore these methods are less time consuming and also less expensive. Effervescent powders also present no concerns about leakage. However, more studies are needed to evaluate if an effervescent inhalable powder can increase the absorption and bioavailability of drugs in the lungs due to improved drug dissolution properties.

4.7 Cough Machine Experiments

The recorded pattern (Figure 4.15 and Figure 4.16) of mucus from the cough machine clearly showed differences between control Mucus Simulant (MS) and MS treated with effervescent powders. Similar results were observed when MS with different degrees of viscosity and elasticity was used. Factors such as concentration of locust bean gum and the addition of sodium tetra borate (STB) influenced the pattern. However, the control showed that in most of the cases, large deposits and some big clusters of deposits on the recorded pattern were observed. When effervescent powders were used, the MS showed a larger amount of smaller deposits. Fewer clusters were observed using the effervescent powders. These graphs generally showed more, smaller points. These results showed that effervescent powders were able to disperse the MS and make the MS less adhesive. Such properties might be used therapeutically to loosen mucus in different sections of the respiratory tract. This study was only descriptive and not quantitative.

Figure 4.15 and Figure 4.16 show the recorded pattern of the mucus from the cough machine using the control mucus simulant and the mucus simulant treated with effervescent powders.

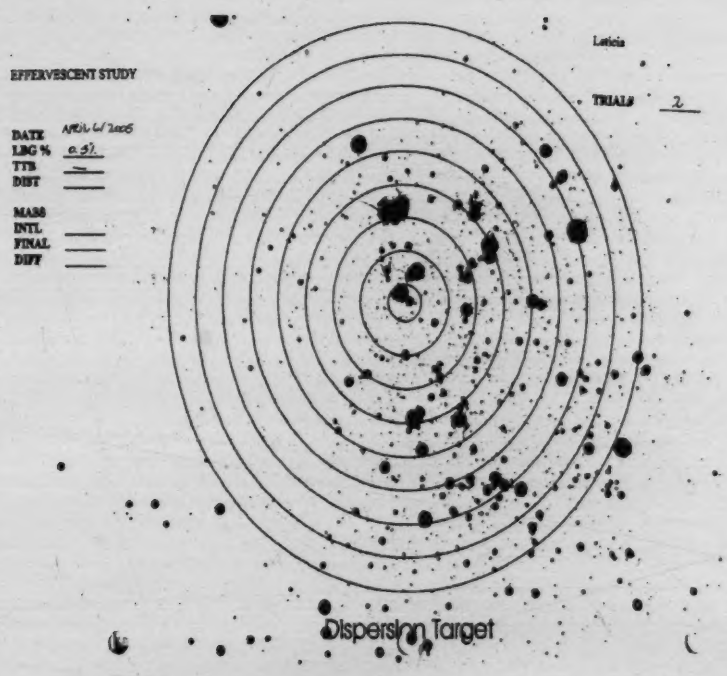


Figure 4.15 Recorded pattern of the control mucus simulant from the cough machine

Figure 4.16 Recorded pattern of the mucus treated with the effervescent powder from the cough machine

4.7.1 Discussion

The effervescent powder was tested using a cough machine. The objective of this experiment was to analyze the effervescent powder properties using the simulated mucus. Mucus is a nonhomogeneous, viscoelastic fluid containing carbohydrates, proteins and lipids in watery matrix (Houtmeyers et al, 1999). Most of the respiratory tract in healthy people is coated with a very thin lining of mucus. Patients with cystic fibrosis or chronic bronchitis for example, are characterized by an increase in the quantity of mucus in the respiratory tract. In diseases that are characterized by mucus hypersecretion and impaired airway clearance, elimination of the excess mucus by coughing becomes of paramount importance.

The results on the cough machine have shown that the effervescent powder was able to make the mucus less adhesive and also decrease the amount of mucus agglomerates. Possible properties of the effervescent powder found in this study were: 1- enhances loosening 2- causes thinning 3- improves cleansing 4- removes mucus from the inner surface of the nose, mouth, airway, and/or lungs of a patient. The properties of the effervescent powder combined with an antibiotic, such as ciprofloxacin, can improve the treatment of patients with cystic fibrosis. It also opens the door for using the effervescent carrier particles with other drugs. These carrier particles have an advantage over simple carrier particles because they agglomerated less and potentially could increase the drug release.

During the coughing the mucus transport in the airways is determined mainly by its surface morphology and the rheological characteristics of the mucus. An example of a rheological characteristic is the viscoelastic material characterized by nonlinear and time dependent flow (Houtmeyers et al, 1999). This is characterized by the thickness of the mucus, the thickness and viscosity of the serous layer fluid and the degree of air flow interaction (Agarwal et al, 1994). If the effervescent powders are able to change the properties of the mucus it may help to increase mucus transport as well.

CHAPTER 5

Conclusion

One of the objectives of this research was to evaluate the influence of the spray dryer parameters and formulation composition of the carrier particles. It was found that the machine parameters as well as the ingredients tested in our formulations had a major effect on the MMAD, FPF, GSD, morphology and shape of the carrier particles.

A new formulation was developed for use in the pulmonary route of administration. This formulation contained effervescent properties with lubricant excipients. The effervescent material added an active release mechanism to the formulation. This mechanism increased drug dissolution and enhanced the dispersion of nanoparticles over the effervescent gas bubble interface. These carrier particles can be synthesized with an adequate particle size for deep lung deposition. These findings showed that the objectives set in the beginning of the project, were achieved.

Furthermore, effervescent carrier particles can be used to deliver a large range of substances to the lungs with possibly a faster release compared to conventional carrier particles. The results (smaller MMAD and higher FPF) showed that potentially effervescent carriers are a better carrier for poorly soluble substances and drug delivery systems, e.g. nanoparticles. For the first time, an effervescent formulation

was developed in order to be applied to the pulmonary route of administration. Results obtained in this project proved our hypothesis that it was possible to produce an inhalable effervescent carrier system that is superior drug delivery system for nanoparticles and drugs compared to the conventional lactose carriers.

Further studies are required in order to evaluate how the effervescent particles will behave at the lung surfactant air interface.

CHAPTER 6

Future work

The study reported here has just opened the doors for a new platform of pulmonary delivery. Some of the findings in this project still need more investigation.

- The dissolution properties of the effervescent carrier particles require more studies. It will be necessary to investigate if the effervescent powders are able to increase the dissolution of drugs in the lungs.

- In order to investigate the dissolution of the effervescent carrier particles, it will be necessary to create a new method of dissolution test.

- Further studies are required to evaluate how the effervescent particles will behave at the lung surfactant air interface.

- Animal studies are necessary in order to prove that the effervescent powders are better carriers of nanoparticles and drugs than lactose carrier particles.

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